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The Students Handbook to the Science of Milk and Milk Products

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THE CHEMISTRY OF MILK AND MILK PRODUCTS

By
L. T. Lowe, B.Sc., F.R.I.C., N.D.A.

SECTION I.—RAW MILK

CONSTITUENTS OF MILK

The milk of a number of different species of animals is used as human food. Besides cows' and goats' milk that from sheep, camels, buffaloes, mares and llamas has been used. Cows' milk, however, represents the preponderant type and the following pages refer only to this kind. The colour may vary from a bluish-white tint to yellow depending on its composition and particularly on the amount of yellow pigment (carotene) present. This in turn depends partly on the breed of cow and partly on its food. Milk fresh from the cow has a characteristic smell, but this should disappear on standing. Any pronounced odour or taste other than faintly sweet is due to a taint.

Milk is a mixture of a number of constituents the proportions of which vary considerably. No definite figures can be given even for its average composition as analysts in different parts of the world report varying results. This is illustrated by the percentages below :—

	Droop Richmond (English samples)	*Tocher (Scottish samples)*	*Van Slyke (American samples)*
Fat	3.75	3.95	3.9
Solids-not-fat	8.91	8.86	9.0
including :—			
Milk Proteins	3.46	3.52	3.2
Lactose	4.70	4.64	5.1
Mineral Matter	0.75	0.70	0.7
Water	87.34	87.19	87.1

MILK FAT

Milk fat (butter fat) is the most valuable constituent from the commercial standpoint, but as regards nutritive value the proteins and mineral matter are probably of even greater importance. It consists of minute globules suspended in the milk "serum". It is now thought that each globule is enclosed in a very thin film of protein and that this film assists in keeping the globules suspended and in preventing their coalescing. Thus it can be shown that the fat cannot be extracted from milk by means of the usual fat solvents—such as ether—until this film has been broken down. The film appears to

contain also lecithin, a substance present in small amounts in milk fat. The globules vary in diameter from 0.0001 mm. to 0.01 mm., the average being about 0.003 mm. (approximately 1/10,000 inch). A single drop of milk contains about 100,000,000 fat globules. The size of the globules is important commercially. Milk with large globules *creams* more rapidly than that with small. Even mechanical separation will not remove the smallest globules. Again cream with large globules churns more easily in buttermaking. Granules of butter may also appear in milk after transport, especially if the globules are large, but this may be prevented by filling the churns completely so as to check the agitation of the milk. In cheesemaking small globules are preferred as large ones rise in the milk in the vat before the clotting brought about by the rennet is able to incorporate them in the curd; thus there is more loss of fat in the whey. Jersey cows produce milk with the largest globules, Friesians the smallest and Ayrshires the next smallest. In each breed the globules gradually become smaller as the lactation advances and also when dry foods are given.

Milk fat is lighter than water, its specific gravity being about 0.91 to 0.92. It melts at 29° to 36°C depending on the relative proportions of hard and soft fats present, and solidifies at 19° to 24°C. It is derived from the fats and carbohydrates of the cows' food, but mainly from the carbohydrates, and provides about half of the "Calorie" value of milk in nutrition. Milk with 3% fat provides about 547 Calories per quart and that containing 5% fat about 778 Calories.

Like other animal and vegetable fats milk fat is a mixture of "glycerides" which are compounds of glycerine with fatty acids. Its average composition is given in *Fig.* 1.

PERCENTAGES OF VARIOUS GLYCERIDES IN MILK FAT.
(Figures due to Winter Blythe).

Name of Glyceride.	*Percentage in milk fat.*
Butryin	3.8
Caproin	3.6
Caprylin	0.5
Caprin	1.8
Laurin	7.4
Myristicin	20.2
Palmitin	25.1
Stearin	1.8
Olein	35.8

Fig. 1.

Milk fat contains higher proportions of glycerides of the lower fatty acids—butyrin, caproin and caprylin—than is the case with other fats. This provides a chemical method of distinguishing butter from margarine. Some foods increase the proportion of palmitin and thus harden the butter fat while others increase the olein and so soften the fat. This is indicated in *Fig.* 2.

EFFECT OF FOOD ON THE HARDNESS OF MILK FAT.

Foods which increase the percentage of palmitin and thus harden the fat.	*Foods which increase the percentage of olein and thus soften the fat.*
Hay (if much clover is present the fat is somewhat softer)	Pasture
Oat straw	Green fodders
Silage	Linseed and Linseed Cake
Barley	Soya Bean Cake
Peas, beans and vetches	Sesame Cake
Groundnut Cake	Sunflower Cake
Coconut Cake	Maize Gluten Meal.

NOTE :—An increase in the proportion of butyrin also softens the milk fat. This glyceride has a lower melting point than olein. Thus cottonseed meal does not soften butter fat because the increase produced in the olein content is counterbalanced by a decrease in butyrin. The effect of some foods on the hardness of milk fat has not yet been determined.

Fig. 2.

Thus on turning out cows to pasture in spring the fat becomes softer and cream for butter-making must be churned at a lower temperature, whereas winter feeding hardens the fat and the churning temperature must be raised. Milk fat readily absorbs odours from the atmosphere, so that milk and milk products must not be stored with strongly smelling substances, such as fish.

Certain chemical changes in milk fat are responsible for taints in milk, cream and butter. Fat-splitting enzymes (*lipases*) may set free fatty acids and so cause rancidity. The olein may combine with oxygen producing a tallowy flavour (*oxidative rancidity*). This change is accelerated by traces of copper. On the other hand certain chemical compounds delay the oxidation of olein, but it is not at present permissible to add any of these to milk or milk products. Chemical changes in fat are illustrated in *Fig.* 3.

DEVELOPMENT OF TAINTS IN MILK AND MILK PRODUCTS THROUGH CHEMICAL CHANGES IN THE FAT.

A. HYDROLYSIS.

$$\text{Glycerides} + \text{Water} \longrightarrow \text{Glycerine} + \text{Fatty Acids.}$$

This change is brought about by fat-splitting enzymes in milk and milk products. Some of the fatty acids (especially butyric acid) thus set free have very rancid flavours.

B. OXIDATION.

$$\text{Olein} \xrightarrow[\text{oxygen}]{\text{in presence of}} \text{Epihydrin Aldehyde.}$$

This " oxidative " rancidity develops more rapidly in presence of traces of metals (particularly copper). Thus a badly worn milk cooler, in which the tin plating has been worn off exposing the copper, may produce this type of taint. This effect is more likely to occur in milk with few bacteria and at low temperatures; under warmer conditions the bacteria use up the oxygen present and so prevent this oxidation.

Fig. 3.

Substances Present in Small Amounts in Milk Fat.

In addition to glycerides milk fat contains small amounts of three other types of substances :—

(i) *Carotene and "Fat Soluble" Vitamins.*

Carotene is the yellow pigment responsible for the colour of butter. It is supplied by green foods, carrots and yellow maize and is the source from which the body manufactures Vitamin A. The change of carotene into vitamin A occurs in the liver. Some breeds—such as Jerseys and Guernseys—transfer much unchanged carotene into the milk and the cream has a "rich" yellow colour. Other breeds—as, for example, Friesians and Ayrshires—change much of the carotene into vitamin A (which is colourless) before transference to the milk, so that the cream is paler in colour. Milk of other species—and human milk—contains little carotene but is well supplied with vitamin A. Similarly the Channel Island breeds store more unchanged carotene in their body fat than do other cattle and the resulting yellow colour in the meat fat is sometimes unpopular with consumers. The carotene content of milk (and hence also the yellow colour of the cream) is increased by feeding green foods which are rich in this constituent. In butter-making the addition of the harmless yellow pigment "annatto" renders these differences commercially of small importance.

Vitamins A and D are present, the latter being more abundant in summer than in winter milk. The Vitamin D content of milk fat can be increased by feeding cod liver oil or irradiated yeast, that is, yeast which has been exposed to ultra-violet light, or by "irradiating" the milk. Milk fat also contains Vitamin E. These three fat-soluble vitamins are resistant to heat and are not destroyed by pasteurisation of milk, but only vitamin A is present in abundance. In infant feeding it is necessary to provide additional Vitamin D in the form of cod liver oil or other fish liver oils.

(ii) *Cholesterol and Ergosterol.*

Cholesterol is characteristic of animal fats. Vegetable fats contain a similar but distinct substance called "phytosterol" the presence of which is sometimes used as a test for indicating admixture of butter with margarine. Cholesterol contains a trace of a related substance—dehydro-cholesterol—which is changed into Vitamin D by ultra-violet light from the sun or carbon-arc or mercury vapour lamps.

(iii) *Lecithin.*

This substance belongs to the group of "phosphorised fats" which are relatively abundant in the blood. In addition to the chemical elements present in true or neutral fats, *viz.* carbon, hydrogen and oxygen, it contains also nitrogen and phosphorus. Decomposition of lecithin may produce "trimethylamine" a substance of intensely

fishy taste and smell and so lead to a " fishy " taint in butter and in dried milk products. Feeding of excessive amounts of sugar beet leaves and crowns or of molassed sugar beet pulp may also cause the formation of trimethylamine and the fishy taint, but in this case decomposition of " betaine " present in sugar beet products is the cause.

The usual methods of determining the fat in milk do not distinguish these substances from the " true " fat so that fat percentages usually quoted include very small quantities of all of the above compounds.

MILK PROTEINS

The muscles and tissues of animals are composed largely of proteins and hence these are commonly referred to as " body building " constituents. Proteins are made up of amino-acids which may be regarded as the essential " building stones " for muscles, nerves and tissues. Some amino-acids cannot be produced by the body and must be supplied in the food. Thus a protein rich in the essential amino-acids has a nutritive value superior to one poor in these constituents. This nutritive superiority is expressed by stating that the superior protein has a higher " biological value." Milk proteins are of very high biological value and rank (along with egg proteins) above those of other foods. This superiority in type of protein combined with the favourable balance of mineral constituents supplied by milk is of great nutritional significance and is the main basis of the high priority accorded to milk production and of the " milk in schools " scheme. The percentage of total proteins in milk does not vary to the same extent as the fat percentage, but breeds of cattle which produce milk rich in fat supply also slightly more protein in their milk than other breeds.

Casein.

This protein does not occur in plants or in animal products other than milk. When pure it is white and without taste or smell. It occurs in milk in " colloidal " solution, that is, in the form of minute particles (about 0.0002 mm. in diameter) which are, however, much larger than the " molecules " and " ions " present in a " true " solution. The white colour of milk is due to the scattering of light by the casein particles; there is no white pigment present. As with other colloidal solutions the particles can be removed by passing milk through a porcelain filter or an animal membrane. *Fig.* 4 illustrates some properties of colloidal solutions.

COLLOIDS AND COLLOIDAL SOLUTIONS.

Suspensions of solid particles in a liquid (or emulsions of liquid globules in another liquid), colloidal solutions and true solutions are distinguished by the sizes of the particles of the " dispersed phase " (*i.e.* the material suspended or dissolved)

Thus :—

	Size of Particles.
Suspensions and Emulsions.	Above $\frac{1}{10,000}$ millimetre.
Colloidal Solutions	$\frac{1}{1,000,000}$ to $\frac{1}{10,000}$ millimetre.
True Solutions	Less than $\frac{1}{1,000,000}$ millimetre.

In milk the fat is present as an emulsion, the proteins and also calcium phosphate as colloidal solutions and the lactose together with about one-third of the mineral matter as true solutions.

In true solutions the particles are " molecules " or " ions " whereas in colloidal solutions they are aggregates of molecules. On evaporating the solvent true solutions leave crystals of the dissolved material, colloidal solutions leave a jelly (a " colloid.")

The particles in colloidal solutions carry a positive or negative electric charge (*e.g.* clay is an " electronegative " colloid). When this charge is neutralised the colloid precipitates as a clot. This occurs at a definite degree of acidity (pH). This point is called the " isoelectric point." The " isoelectric point " of casein is at pH 4.6.

Fig. 4.

Casein is present in combination with calcium. When an acid is added, or when acidity is developed through souring of milk, the acid removes the calcium and the casein clots. Boiling slightly sour milk or the addition of a mixture of alcohol and water hasten the clotting, and can be used as tests for souring. Casein in fresh milk is not clotted by heat except when milk is heated under pressure to temperatures above its normal boiling point. Casein, is, however, present in the surface skin which forms when fresh milk is boiled.

In cheese-making the enzyme *rennin* from the rennet converts the casein into *paracasein* which coagulates and entangles the milk fat. This clot is not identical with that obtained through the action of acids; it is much softer and more plastic. Rennin is present in the stomachs of young suckling animals, its purpose being to produce an easily digested clot. In the adult stomach casein is coagulated by the digestive enzyme *pepsin.*

Casein is prepared mainly from separated milk by the addition of rennet or dilute acids. It has a great variety of uses in industry including the manufacture of infant and invalid foods, of non-inflammable plastics and fibre, of paints, distempers and glues, of " spreaders " for inclusion in sprays for fruit trees and of materials for imparting a " gloss " to paper. Casein plastics are used as insulators in electric circuits, for billiard balls and for many domestic articles, such as buttons and combs. Casein glues are employed in the manu-

facture of furniture, motor cars, aeroplanes and refrigerators. Casein paints are used for walls and for outdoor purposes. Casein fibre finds uses in the manufacture of hats. By far the greatest use of casein is however, in the making of high-grade paper.

Lactalbumin.

This protein is similar to that of white of egg (*ovalbumin*) but is not identical with it. Unlike casein it is not clotted by rennet and thus passes into the whey during cheese-making. It clots on heating and is present in the "skin" which forms on boiled milk and in the film produced on milk utensils when these are steamed without previous adequate washing in cold water. Lactalbumin finds little industrial use, but it is an important constituent of dried whey.

Lactoglobulin.

Although present only in traces in milk this protein is abundant in "colostrum," the first milk after calving. The high acidity of colostrum and also its clotting on heating are due to this constituent. Lactoglobulin contains a high proportion of the amino-acid "proline" necessary for blood formation and is associated with various "antibodies" which protect the offspring from infection. Substances checking putrefaction in the intestines are also present in it. It is not clotted by rennet.

LACTOSE (MILK SUGAR)

Lactose occurs only in milk. It is manufactured in the milk gland from the glucose (*dextrose*) of the blood. The percentage present — usually 4.8% — varies very little in normal milk, but is much reduced in milk from udders affected with mastitis organisms. It is only about one-sixth as sweet as cane or beet sugar (*sucrose*). It provides about 30 per cent of the "Calorie" value of milk. It is present in "true" solution and thus, unlike the fat and proteins, affects the freezing point of milk.

When milk sours the lactose is converted by bacteria into lactic acid. Most bacteria are inactive below 50° F so that efficient cooling of milk delays souring. A sour taste is usually apparent when milk has about 0.4% acidity; clotting takes place at about 0.7%. When the acidity reaches 1% the souring bacteria are checked and no further acid is produced. At this stage about 30% of the lactose has been converted into lactic acid. If the acidity is reduced by the addition of an alkali such as lime, further fermentation of lactose to lactic acid takes place. The lactic fermentation is of great importance in "ripening" cream for butter-making and in cheese-making. It is also used in preparing special sour milk drinks such as Kefir, Koumiss and Yoghourt. In the manufacture of the above dairy products lactic acid serves a subsidiary, but essential, purpose by checking the growth of organisms which would cause undesirable taints. A large number of

species of bacteria form lactic acid from lactose, but only those are desirable which do not in addition form gases and traces of other acids. The presence of desirable types is ensured by the use of " pure culture " starters.

The charring of milk on heating is largely due to the formation of " lacto-caramel " from lactose. Lactose is obtained chiefly from whey and is used in the preparation of infant and invalid foods.

MINERAL MATTER (ASH)

Milk contains all the mineral elements found in the animal body and its correct balance of these contributes to its importance in nutrition. Thus an important part of the value of skimmed milk and whey for pigs lies in their mineral content and their power of making good the mineral deficiencies of cereal meals. Approximately one-third of the mineral matter is present in " true " solution. The remainder is associated with the milk solids. Calcium, phosphorus and sulphur are combined with the proteins. Milk is very poor in iron. The young animal relies for this element on reserves present in the body at birth. Milk from cows affected with " mastitis " contains an abnormal proportion of common salt. The average composition of milk ash is given in *Fig.* 5.

AVERAGE COMPOSITION OF MILK ASH.
(Figures from the Rowett Research Institute).

The following table gives the percentages of the mineral elements in milk ash, but does not indicate how they are combined in the original milk. The relative proportions of the mineral elements are of importance in the manufacture of evaporated milk (unsweetened condensed milk) as they control the " thickness " of the product.

	Percentage of the ash.
Potash (K_2O)	26.8
Soda (Na_2O)	10.1
Lime (CaO)	22.0
Iron Oxide (Fe_2O_3)	0.02
Chlorine (present in the form of chlorides)	14.7
Phosphoric Acid (P_2O_5)	30.4
Citric Acid	Traces
Magnesia (MgO)	Traces

The chief inorganic compounds in milk ash are phosphate of lime and the chlorides of sodium and potassium.

Fig. 5.

OTHER SUBSTANCES OCCURRING IN MILK

Dissolved Gases.

Freshly drawn milk contains carbon dioxide, oxygen and nitrogen. The carbon dioxide comes from the udder while nitrogen and oxygen are absorbed during milking. The total gases in freshly drawn milk

amount to 7%-10% by volume. On standing carbon dioxide is lost. This loss causes a slight increase in the specific gravity of milk; this increase is known as "Recknagel's phenomenon." Certain bacteria present in milk obtained under dirty conditions also produce gases, but these are not apparent until the milk has stood for several hours. This effect is more pronounced in hot weather.

Water-soluble Vitamins.

Milk contains all the water-soluble vitamins but is poor in Vitamin C so that alternative sources, such as orange or blackcurrant juices, are needed in infant feeding. This vitamin is easily destroyed by exposure to heat and to sunlight, and disappears when milk in bottles is exposed to the sun. The vitamins of the "Vitamin B" group are relatively heat resistant. One of them, "riboflavin," imparts the greenish-yellow colour to whey. This pigment, formerly known as "lactoflavin" or "lactochrome," is masked in milk by the white colour. It occurs in the milk of most species but the quantity present, unlike that of carotene, is not affected by the food of the animal. Enzymes also occur in milk. These are described in detail later.

VARIATIONS IN THE COMPOSITION OF MILK

The proportions of all the constituents of milk are subject to variation. This is of considerable importance in connexion with legal proceedings in cases of suspected adulteration. English law provides that nothing shall be added to or removed from cows' milk offered for sale. The Sale of Foods and Drugs Act, 1938, condemns the addition of any of the following substances:—water, dried milk, separated milk, condensed milk, colouring matter and preservatives. This Act empowers the Minister of Agriculture to lay down minimum standards of composition for milk and separated milk. These standards form a basis upon which a presumption of adulteration may be founded. The Sale of Milk Regulations, 1939, state that if a sample of milk is found to contain less than 3 per cent of fat or 8.5 per cent of non-fatty solids it shall be presumed, unless the contrary can be proved, to be adulterated. For separated milk the minimum limit of composition is 8.7 per cent of non-fatty solids.

Although the above figures are well below the average composition, genuine milk on occasion falls below them. Probably this does not happen in more than 1 per cent of samples but the possibility of unjust conviction for adulteration remains. It is thus important that the dairy farmer should be aware of the factors which tend to lower the percentages of fat and of non-fatty solids and should take appropriate action to eliminate them. Variations in composition are important also in two other connexions. They affect the nutritive value. Further when milk is used for the preparation of manufactured products, variations in its composition affect both the yield and the quality of the product. For this reason manufacturers keep a close watch on the composition of the milk they purchase as well as on its sweetness and

freedom from bacteria and taints. The variations in percentages of the main constituents which may occur in genuine unadulterated milk from herds of healthy cows are as follows :—Fat 2.6%-6.0%, Total Proteins 2.8%-4.0%, Lactose 4.5%-5.2%, Mineral Matter 0.6%-0.8%. Even wider variations than these are occasionally found for individual cows and disease will extend these limits considerably.

CAUSES OF VARIATION IN THE COMPOSITION OF MILK FROM INDIVIDUAL COWS

(1) Individuality of the Cow.

Some cows although perfectly healthy and well-managed persistently produce milk below the legal standards. This is apparently an inherited characteristic and no system of feeding or management will effect the desired improvement. It is unusual, however, for such cows to produce milk *greatly* below the legal standards. The detection and elimination of such animals is nevertheless important, especially in small herds or where the herd milk is not well mixed.

(2) Breed.

Although there is not sufficient information available to permit of an accurate comparison between breeds with respect to the average composition of their milk it is clear that Friesians tend to produce the poorest and Jerseys and Guernseys the richest milk. *Fig.* 6 gives the average results from 6,566 analyses of milk from cows exhibited at forty Annual Dairy Shows held by the British Dairy Farmers' Association.

AVERAGE COMPOSITION OF MILK FROM COWS EXHIBITED AT FORTY ANNUAL DAIRY SHOWS.

(Figures due to T. J. Drakeley and published in The Journal of Agricultural Science, 1927.)

Breed	*Average percentage of fat*	*Breed*	*Average percentage of non-fatty solids*
Jersey	5.18	Jersey	9.30
Guernsey	4.88	Guernsey	9.29
Kerry	4.30	South Devon ...	9.25
Dexter	4.15	Dexter	9.11
South Devon... ...	4.02	Kerry	9.09
Ayrshire	3.97	Red Poll	9.09
Red Poll	3.81	Dairy Shorthorn ...	9.04
Dairy Shorthorn ...	3.78	Ayrshire	9.00
Lincoln Red	3.76	Lincoln Red	9.00
British Friesian ...	3.67	British Friesian ...	8.78

Fig. 6.

(3). Disease of the Udder.

The effect of mastitis is to reduce the percentage of lactose (and thus the total non-fatty solids) and to increase in smaller degree the salt in the mineral matter. These are the two constituents present in "true" solution but the rise in salt counterbalances the fall in lactose leaving the freezing point unaltered. The use of the freezing point test is therefore indicated in doubtful cases of adulteration. Severe mastitis may also depress the fat percentage. *Fig.* 7 illustrates these effects.

EFFECT OF MASTITIS ON THE COMPOSITION OF MILK.

	Ordinary Mastitis (cow recovering from an acute attack)	*Severe Mastitis*	*Healthy Cow in same Herd*
Percentage of fat	4.0	0.3	4.0
Percentage of lactose ...	2.5	Trace only	4.8
Percentage of salt... ...	0.19	0.50	0.14

Fig. 7.

(4) Age.

There is a slight decrease in both fat and non-fatty solids from 3 years onwards. Speir has found a gradual falling off in the fat percentage with Ayrshire cows from 3.87% at 3 years to 3.42% at 13 years, while Tocher has noted a uniform fall in non-fatty solids from 8.9% at 3 years to 8.6% at 13 years.

(5) Stage of Lactation.

In general the percentages of fat and non-fatty solids are both inversely related to the total yield. They decline for some weeks after calving as the yield increases and increase later as the yield falls off. The slight rise in the percentage of non-fatty solids as the cow dries off is due to an increase in protein. The percentage of lactose is unaffected by the period of lactation. The stage at which these percentages reach a minimum does not, however, exactly correspond to that of maximum yield being somewhat later and varying in different breeds between 40 and 100 days after calving. The total variation in fat percentage may reach 1.0 per cent in Guernseys but is smaller in some other breeds, while that in solids-not-fat usually does not exceed 0.3 per cent.

(6) Feeding.

If adequate and *balanced* rations are provided feeding has little effect on the composition of milk, which is determined mainly by hereditary factors. Some variation often occurs when the feeding is changed but such effects are usually only temporary. Rations containing high amounts of oil—as from ground linseed—have occasionally

raised fat percentages very slightly, but excessively oily foods are undesirable. German investigators have claimed that palm kernel cake increases the fat percentage, but English experiments have failed to confirm this. Excessive feeding of cod liver oil or of rice meal may lower fat percentages very appreciably.

Underfeeding affects the yield much more than the composition of milk. The reason for this is that the milk constituents are manufactured in the milk gland from those of the blood and the composition of the latter remains constant, body reserves being drawn on in cases of food deficiencies. Other properties of the milk—such as colour of cream, hardness of fat and some taints—are affected by the food. These effects are described elsewhere.

(7) Intervals between Milkings.

This is by far the most important factor in causing variations in fat percentage. Milking at 12 hour intervals, such as 6 a.m. and 6 p.m., produces least variation; the morning's milk is then very slightly the richer. When, as commonly occurs, the day interval is considerably shorter than the night interval the morning's milk is much poorer in fat and may be below the legal standards. The solids-not-fat percentage is little affected. *Fig.* 8 illustrates this effect. Milking 3 or 4 times daily gives fat percentages slightly higher than those obtained with 2 milkings.

EFFECT OF LENGTH OF INTERVALS BETWEEN MILKINGS.

(Figures due to W. A. D. Rudge, and published in the Annual Report of the Department of Agriculture, University of Cambridge, 1903).

A.M. Milking			*P.M. Milking*		
Length of night interval	*Percentage fat*	*Percentage solids-not-fat*	*Length of day interval*	*Percentage fat*	*Percentage solids-not-fat*
12 hours ...	3.64	8.81	12 hours ...	3.45	8.92
16 hours ...	2.33	8.97	8 hours ...	4.47	8.92

Fig. 8.

(8) Efficiency of Milking.

The strippings contain 8 or 9 per cent of fat and must therefore be included in the milk. On the other hand the first-drawn milk is very poor, containing only about one per cent of this constituent. In general the higher the yield the bigger is the difference in fat content between the first-drawn milk and the "strippings." The percentage of non-fatty solids is not affected.

(9) Excitement.

There are often considerable fluctuations in yield and fat percentage associated with "heat" periods. Immediately preceding the period the milk is often richer in fat, whereas at the first milking during

this period both yield and quality are low. The next milking produces both high yield and high fat content. Fright at milking time or a change of milkers may lower the fat percentage through failure to secure the strippings.

(10) Colostrum.

Milk produced immediately after calving is very high in solids-not-fat owing to the large proportions of lactalbumin and lactoglobulin present. Colostrum is richer also in casein and mineral matter than normal milk, but is poorer in fat and lactose. Its colour is reddish-yellow, it has a bitter "fleshy" taste and abnormal smell. The very high percentage of lactalbumin gives a more acid reaction and increases the specific gravity much above that of normal milk. The fat globules are not spherical and distinct from each other as in normal milk, but occur in irregular masses. There is a gradual change to normal within a week. This is illustrated in *Fig.* 9.

CHARACTERISTICS OF MILK DURING FIRST FEW DAYS AFTER CALVING.

Time after calving	*Specific Gravity*	*Fat Percentage*	*S.N.F. Percentage*	*Albumin + Globulin Percentage*	*Coagulation on boiling?*
Immediately	1.067	5.10	21.89	11.34	Yes
6 hours	1.044	6.85	13.61	6.30	,,
12 hours	1.037	3.80	10.73	2.96	,,
24 hours	1.034	3.40	9.37	1.48	,,
36 hours	1.032	3.55	8.67	1.03	,,
48 hours	1.032	2.80	8.64	0.99	,,
3 days	1.032	3.10	8.76	0.97	No
4 days	1.032	2.80	9.05	0.82	,,
5 days	1.032	3.75	8.92	0.87	,,
7 days	1.032	3.45	8.68	0.69	,,

Fig. 9.

(11) Fatness of the Cow at Calving.

American experiments indicate that if a cow is excessively fat at the time of calving her milk for the first week may contain half as much fat again as is present during the remainder of the lactation (*e.g.* 5%-6% during the first week as compared with an average of 3.4%-3.6% over the whole lactation). Fat percentages fall to the normal figure in 15 to 30 days after calving. Little experimental work appears to have been done in this country on this factor, and good herd management avoids excessive fatness at calving. Misleading figures for milk composition may possibly be obtained if the cow calves down very fat and milk samples are analysed only during the first 1 or 2 weeks.

CAUSES OF VARIATION IN HERD MILK

The mixed milk from a herd should show much less variation than that from a single cow as conditions influencing some animals

may be counterbalanced by other factors affecting other cows. Breed, length of intervals between milkings and efficiency of milking will, however, affect the milk of the whole herd. Some other factors affecting the mixed milk of a herd are considered below.

(1) Efficiency in Mixing Milk.

It is essential to avoid including the milk from several heavy milking cows in the same churn, otherwise this churn may contain milk with less than 3 per cent of fat. This may be effected either by spacing the cows so that heavy milkers are not milked in succession, or by the use of a " distributor " on the cooler to enable several churns to be filled simultaneously.

(2) Herd Yield.

If a large proportion of the cows are in full milk at the same time the herd milk may be low in fat. This deficiency is accentuated if the night interval between milkings is unusually long. As the herd yield falls the fat percentage rises.

(3) Day to Day Variations.

These are greater in small than in large herds and more pronounced in evening than in morning milk. The existence of this variation may make it difficult to prove that milk below the legal standards is genuine, as a sample taken from the same herd on another day of the same week may have more than 3% fat.

(4) Seasonal Variations.

In general the fat percentage tends to be lowest in May, coinciding with the rise in yield on turning out to grass, and highest in November. The extent to which the yield rises on turning out to grass depends on the previous indoor feeding. If this consisted of hay and concentrates the yield on turning out may rise by 10 per cent and the fat percentage will fall considerably. Winter feeding of rations rich in carotene—such as grass silage or dried young grass—much reduce the stimulus of turning out to pasture. The consequent increase in yield is then much smaller and the fat percentage is less affected. Some variation in fat content, however, occurs even if the food is unchanged. The seasonal factor then mainly concerned appears to be temperature. Carefully controlled American experiments showed an average increase of 0.2% in fat percentage for each fall of 10° between 72°F and 27°F. There is a similar, but smaller, variation in the percentage of non-fatty solids. If a high proportion of the herd calve in autumn the high yields in November will reduce the fat percentage during that month.

(5) Weather Conditions.

Long periods of summer drought and the consequent shortage of " keep " reduce yields, raise the fat percentage but depress the solids-not-fat content.

(6) Barren Cows.

A considerable proportion of barren cows in the herd may depress the percentage of non-fatty solids below the legal standards.

PRACTICAL RECOMMENDATIONS FOR HERDS PRODUCING LOW QUALITY MILK

A.—Milk Low in Fat.

(1) Detect and eliminate individual cows giving a low fat percentage in their milk.
(2) Milk at intervals as nearly equal as possible.
(3) Milk heavy yielders last at night and first in the morning to equalise the day and night intervals.
(4) Make sure that milking is efficient and stripping thoroughly carried out. Milk recording will assist in this.
(5) Feed adequate and balanced rations and "steam up" before calving to provide body reserves.
(6) "Space" cows so that milk from several very heavy yielders is not in the same churn. Use a distributor on the cooler.
(7) Take account of fat percentages in selecting the bull and cows for breeding replacements. High fat percentage in milk is a hereditary character.

B.—Milk Low in Solids-not-Fat.

(1) Remove barren cows from the herd.
(2) Control mastitis. The "panel" scheme for dairy farmers will assist by providing regular testing and treatment.
(3) Provide adequate minerals in the ration.
(4) Take account of percentages of non-fatty solids in selecting the bull and cows for breeding replacements.

SPECIFIC GRAVITY OF MILK

Milk is heavier than an equal volume of water. The figure obtained by dividing the weight of milk by that of an equal volume of water (at the same temperature) is called the "specific gravity" and averages 1.032, but variations for herd milk from 1.027 to 1.035 have been recorded. Since milk fat is lighter and the other milk solids heavier than milk an increased percentage of fat lowers the specific gravity while increasing the percentages of other milk solids raises it. The specific gravity also decreases with rise in temperature and the figure is usually corrected to 60° F. Determinations of specific gravity are used for detecting adulteration of milk.

ACIDITY IN MILK AND SOURING.

Milk freshly drawn from the cow does not contain lactic acid but is slightly acid. The pH of fresh milk is usually about 6.5. This *natural* acidity is due mainly to the phosphates and citrates of the ash, the milk proteins and the small amount of carbon dioxide present. On standing lactic acid is developed by bacterial action from the

lactose. This additional acidity through souring can be described as *developed* acidity. Thus the total acidity of milk is made up partly of the " natural " and partly of the " developed " acidity.

Total acidity is estimated by determining the quantity of caustic soda solution (*one-ninth normal* in strength) required to neutralise it. The result is usually expressed as a percentage of lactic acid although the latter is actually present only in the developed acidity.

The natural acidity of freshly drawn milk varies with different cows. The average (expressed as a percentage of lactic acid) is about 0.16% but is higher in early lactation and lower when the cow is nearly dry. Colostrum has a much higher natural acidity amounting to about 0.4% (expressed as lactic acid) due to its high proportions of lactalbumin and lactoglobulin.

Developed acidity will cause clotting of milk when heated. For this reason it is usual to determine the total acidity of milk entering pasteurising and drying plants and factories making condensed and evaporated milks, and to reject consignments with total acidities over 0.19 per cent. This may possibly be unfair to producers whose milk has a high *natural* acidity, but no simple routine test for distinguishing natural from developed acidity is at present available. High natural acidity is often associated with a high percentage of non-fatty solids.

TAINTS IN MILK.

Commercially the keeping quality of milk and its freedom from taints are even more important than its chemical composition as they are more obvious to the consumer. The pleasantly sweet flavour of fresh milk depends on the percentage of lactose being maintained and on the salt in the mineral matter not being abnormally high. Milk low in lactose and unusually high in chlorides has a salty flavour; this may occur when the cow is nearly dry or is recovering from mastitis. All the constituents are liable to chemical changes which may adversely affect the flavour. Taints are often transient being caused by a particular combination of circumstances and disappearing when this is altered. It is convenient to group them according to their origin rather than in accordance with the chemical changes involved.

Physiological Effects.

Some cows normally produce milk with a " flat " unattractive flavour which is quite independent of external conditions, but cases of this kind are few. During the first few days after calving milk may have a bitter taste; when the cow is nearly dry, a sharp salty flavour may develop. Digestive disturbances may also produce " off " flavours as also the prolonged feeding of rations deficient in calcium and phosphates.

Effects of Disease.

During attacks of acute mastitis the milk may have a bitter taste; after recovery a salty flavour may remain for some time. Catarrhs of the udder may also produce unpleasant flavours.

Food and Medicines.

Pasture, good hay, oats, carrots, rice meal and malt culms improve milk flavour. Sugar beet pulp, crowns and leaves may produce a "fishy" taste if more than 7 to 8 lb. of pulp or 50 to 60 lb. of leaves per head daily is fed. Molassed beet pulp is particularly liable to cause this trouble. The feeding of silage or of excessive quantities of cabbages, turnips or swedes (particularly if partly decayed) immediately before milking is very liable to taint milk. These foods should, if possible, be fed *after* milking and in any case not less than four hours prior to milking. Excess kale may give a sickly taste. Rape cake gives a sharp astringent taste and is also a dangerous food. Mouldy cakes and meals, in addition to their liability to cause poisoning, may taint milk.

Cows under veterinary treatment may produce milk tainted by various medicines particularly chlorodyne, iodine compounds and aloes. The last-named produces a strong bitter flavour.

Weeds.

Garlic, garlic mustard (*Jack-by-the-hedge*), and pennycress cause a strong onion-like flavour. Mayweed in hay may produce a "tarry" taste. This plant does not appear to taint milk if fed green; moreover the constituent concerned is volatile and gradually disappears from hay so that hay over six months old does not cause trouble. A large number of other weeds are suspected of milk tainting but the evidence is inconclusive. The chief of these are shepherd's purse, wood sorrel, wormwood, butterwort and tansy.

Absorbed Flavours.

Milk will absorb flavours from any strongly smelling substance, the flavours being apparently carried on the fat globules. Dung, stale food and disinfectants are the main causes of trouble in this connexion. Flavours are most easily absorbed when the milk is exposed in thin layers as on the cooler. Milk should be removed from the cowshed as soon as possible and the cooler should be used in a separate room. Similarly milk should not be stored in a refrigerator along with strongly smelling foods such as fish or bananas.

Bacterial Infection.

This is much the commonest cause. Taints caused by bacteria and other micro-organisms, including yeasts, develop slowly as distinct from those due to other causes which are apparent when the milk is first drawn and do not increase on standing. Infected water supplies, dusty foods and unsterile utensils are common sources of bacterial taints which include "ropy" or slimy milk, "gassy" milk and "burnt," "doughy" and "chocolate-like" flavours. Some types of tallowy and fishy flavours are also due to micro-organisms. Bacterial taints are commonest during the spring and early summer, but attention to the well known recommendations for the production of "clean" milk of low bacterial content should prevent troubles of this kind.

Flavours Due to Metals.

The commonest of these is the "tallowy" or "cardboard" taint brought about by traces of copper and described earlier.

Effects of Pasteurisation and Sterilisation.

The Holder process of pasteurisation in which milk is maintained at 140°-145°F for 30 minutes improves the flavour by dissipating absorbed taints. Flash pasteurisation in which heating to about 160°F for a few seconds is used may impart a slightly "cooked" taste due to the effect of heat on the lactose and lactalbumin. This effect is more pronounced in sterilised milk which has been heated above the boiling point of water.

THE FREEZING POINT OF MILK.

Milk has a slightly lower freezing point than water. This is due to the substances present in true solution—the lactose and the water-soluble portion of the mineral constituents. Substances, such as milk fat, present as emulsions in water or milk proteins, present as colloidal solutions, are without effect on the freezing point. Since the percentages of lactose and of soluble mineral matter are relatively constant so also is the freezing point. Thus freezing point determinations can be used to determine the presence of added water. The freezing point of unadulterated fresh milk lies within the limits of — 0.53°C and — 0.57°C with an average of — 0.55°C. Provided that nothing has been added variations from the normal composition have little effect on the freezing point. This is true even in cases of mastitis infection for the decrease in the percentage of lactose is accompanied by a compensating increase in that of soluble mineral matter.

While the addition of water raises the freezing point, that of materials soluble in water (*e.g.* preservatives) lowers it. Similarly when milk sours the freezing point is lowered as the lactic acid produced increases the percentage of soluble mineral constituents by bringing some of the calcium combined with the casein into a water-soluble form.

Freezing milk affects its flavour. Such milk after thawing has a "watered" taste; also the volume of cream which the milk will produce on standing is much reduced. These effects appear to be due to the rupturing of some of the fat globules on freezing with an effect somewhat similar to that of churning.

VISCOSITY OF MILK.

The viscosity of a fluid is a measure of the difficulty of pouring it. Thus treacle and glycerine, which are difficult to pour, are viscous liquids, that is, their viscosity is high. Milk is about 1.5 to 1.7 times more viscous than water. Heating lowers its viscosity. It is partly for this reason that milk is warmed prior to running it through a cream separator. The resulting lowered viscosity makes the separation of fat easier. On the other hand, to increase viscosity in cream and in the making of ice-cream the cream is held at a low temperature for 12 to

48 hours. High viscosity is desirable in these products and also in condensed and evaporated milks as it gives an impression of richness. Heating milk under pressure above its boiling point also increases its viscosity. This happens during the final sterilisation of evaporated milk in the tins. These increases in viscosity (and also that due to incipient souring) are due to changes in the milk proteins, especially in the casein.

Milk possesses considerable adhesive properties which are due to the casein. Casein glue is one of our strongest glues.

WASHING OF EQUIPMENT.

Thorough cleansing of dairy equipment is essential before sterilisation. Hot water alone is unable to remove films of fat and protein from milk utensils. Washing powders (*detergents*) are therefore used. The cleansing agents employed in such powders are alkalies such as sodium carbonate in the form of either soda ash or washing soda, caustic soda, sodium metasilicate and trisodium phosphate.

A satisfactory detergent must have the following properties :—

(1) It must thoroughly wet the film on the utensils.
(2) It must emulsify the fat in the film, breaking it up into tiny droplets easily washed away.
(3) It must dissolve milk proteins in the film.
(4) It must soften hard water so that a troublesome deposit is not produced if hard water has to be used.
(5) It must rinse off from the surface readily.
(6) If the utensils are tin-plated or are of aluminium the detergent must contain a substance to prevent corrosion of the metal by the soda.
(7) If the utensils are to be washed by hand the detergent must not injure the skin.
(8) It should have some power of destroying bacteria. This is less important for milking buckets and milk churns than for the washing of bottles by machinery.

Caustic Soda has considerable germicidal power, softens water and dissolves protein effectively, but it is not a good emulsifying agent for milk fat. It is difficult to rinse off, its wetting power is poor, and it is very corrosive to metals, especially aluminium. It is also very corrosive to the skin; solutions for hand washing must not contain more than 0.25% of caustic soda. It has thus only a very restricted use in washing powders.

Soda Ash and Washing Soda are cheaper than caustic soda and less corrosive both to metals and to the skin. They do, however, attack aluminium. They are good water softeners, they dissolve protein and emulsify fat well, but their wetting power is poor and they do not rinse off well. Soda ash is the main constituent of most dairy detergents but other materials are added to improve its wetting and rinsing properties and to prevent corrosion of equipment.

Sodium Metasilicate is included to increase wetting power and so facilitate penetration of the film of protein and fat. Moreover it reduces

the risk of blockage of jets in washing machines by the material removed from the films. *Trisodium Phosphate* is included to improve rinsing properties.

In addition to the above substances others are included in special circumstances. *Sodium hexametaphosphate* (sold under the trade name of *Calgon*) is included when hard water is used. *Sodium perborate* is employed to prevent corrosion of aluminium utensils. *Sodium sulphite* is sometimes included to check corrosion of tin plating.

Examples of Detergent Powders.

A commonly used cleaning powder contains:—60% soda ash, 25% trisodium phosphate and 15% sodium metasilicate. For milking machines a common mixture is:—60% soda ash, 10% trisodium phosphate, 20% sodium metasilicate and 10% sodium sulphite. For greasy floors soap is included; a suitable formula for this purpose is:—70% soda ash, 10% trisodium phosphate, 15% sodium metasilicate and 5% soap flakes.

New Types of Detergents.

Experiments in the use for dairy equipment of the new "non-fatty soaps" are being made. These are produced from petroleum (they are "sulphonated olefines") and are not affected by hard water.

STERILISATION OF UTENSILS.

Steam sterilisation is to be preferred to chemical disinfectants as steam has much more penetrating power. Disinfectants may be employed provided that utensils are first thoroughly cleansed and that the correct concentration of disinfecting agent is used. Although many chemical compounds kill bacteria, only those which liberate chlorine gas are at present in use for dairy equipment. Of the latter sodium hypochlorite is the most convenient and is commonly purchased as a stock solution containing 10 per cent of "available chlorine." This means that 100 lb. of the solution will provide 10 lb. of chlorine gas. This gas is the effective sterilising agent. Care must be taken to avoid any trace of hypochlorite passing into the milk as this would contravene the Food and Drugs Act.

The following directions if strictly followed will give effective sterilisation of utensils without contaminating the milk and can be recommended where steam sterilisation is not possible.

(1) Wash the utensils inside and out with cold water immediately after milking.

(2) Make up a "chlorine wash" by dissolving ½ lb. of washing soda and ¼ pint of stock hypochlorite solution in 10 gallons of water. Heat this solution to 105°-110°F and scrub the utensils vigorously in it, keeping them well immersed for at least one minute.

(3) Rinse the utensils in a bath of "chlorinated water" prepared by adding 1 large teaspoonful of stock hypochlorite solution to 10 gallons of water. Allow to drain and dry in a clean place. Utensils must be quite dry before use.

(4) Once weekly thoroughly scald the utensils with water at 180°F.

For aluminium utensils a cleansing agent containing sodium metasilicate should replace the washing soda.

USE OF METALS FOR DAIRY EQUIPMENT.

The following factors have to be considered in the choice of metals for dairy equipment :—

(1) *Cost.* This dominating factor often precludes the use of metals—such as stainless steel—which on other grounds would be admirable.

(2) *Resistance to Mechanical Wear.* This factor is of great importance for milking buckets and milk churns owing to their constant handling. For such equipment this factor combined with that of cost restricts choice to tinned steel.

(3) *Lightness in Weight.* Unfortunately the only light weight metal which can be cheaply manufactured—aluminium—does not withstand rough handling nor is it resistant to corrosion by soda.

(4) *Resistance to Corrosion.* Four different types of corrosion have to be considered, *viz* :—corrosion by milk, sour milk or milk products such as whey, by washing powders, by hypochlorites if these are used for sterilisation, and in refrigerators by the brines used. The relative resistance of the various metals to these distinct corroding agents is considered below.

(5) *Thermal Conductivity.* For equipment in which milk or milk products are to be heated or cooled—such as milk coolers, cheese vats, pasteurising plants and evaporating pans for the manufacture of condensed milk—the conduction of heat by the metal is of importance. Of the common metals copper has the highest thermal (and also electrical) conductivity.

Copper and Iron are unsuitable as they are quickly corroded by milk and the milk dissolves some of the metal. Copper is extensively used for equipment in which milk is to be heated or cooled, but is always tinned over to prevent corrosion. Galvanised iron (iron coated with zinc) is unsuitable as zinc readily corrodes and is poisonous. *Ordinary steels* are quickly corroded by milk, but tin-plated steel is the cheapest and most commonly used material for milking buckets and churns. Tinned utensils are slowly attacked by all the alkaline detergents, but may be protected by including sodium sulphite in the washing powder. For this purpose 1 part of sodium sulphite is added to 10 parts of washing soda or to 4 parts of soda ash. Sodium chromate will also protect tin against alkalies, but causes dermatitis (skin troubles) on the workers' hands. *Stainless steels* are resistant to corrosion both by milk and by cleansing agents but are relatively expensive; also they are poor conductors of heat. These steels are alloys of iron with nickel and chromium. There are 3 main types, viz :—(*a*) containing 13-14% chromium but no nickel. (*b*)

containing 20% chromium and 2% nickel; (*c*) containing 18% chromium and 12-13% nickel. Both (*a*) and (*b*) are magnetic, but (*c*) is not. The last type is also more ductile and hence more easily worked. For very high resistance to corrosion (*e.g.* for use with vinegar) 3% molybdenum is also included. Both tinned steels and stainless steels—particularly the latter—are more resistant to corrosion by hypochlorites than other metals.

Aluminium will resist corrosion by sweet or sour milk, but it is readily corroded by the alkalies used in washing powders, by hypochlorites and also by refrigerator brines. Sodium silicates corrode aluminium less than caustic soda or washing soda. Corrosion is reduced by including sodium perborate in the detergent. If aluminium is used in dairy equipment care in the selection of washing powders is very necessary; both sodium silicate and sodium perborate should be included in these. *Lead* is very unsuitable for dairy equipment as it is easily attacked and is very poisonous. Moreover, lead is a cumulative poison. *Chromium-plating* is unsuitable as it easily peels off.

Wood is used where resistance to dilute acids is important, as in butter-making and in the manufacture of casein. It is difficult to sterilise effectively. Hard woods, such as oak and beech, are the most satisfactory as they are the least absorbent.

Effect of Metals on Milk.

Metals combine with the milk proteins. They may impart a metallic flavour (especially iron and zinc) or may produce oily taints through oxidation of the "olein" in the milk fat (especially copper).

ENZYMES IN MILK.

A number of enzymes (ferments) occur naturally in milk while others are produced by milk bacteria. These are of importance as they cause chemical changes in the milk constituents. Use is made of some of them in testing for efficiency of pasteurisation and for freedom from gross bacterial contamination.

Enzymes Naturally Present in Milk.

(1) *Fat-splitting Enzymes* (*Lipases*).

These produce rancidity by splitting the fat and setting free fatty acids. Normally they are kept in check by the lactic acid bacteria, but rancidity of this type may occur in butter made from unpasteurised cream, in cream ripened at low temperatures and in condensed milk made without previous pasteurisation. Traces of copper prevent lipase action. Lipase activity also occurs occasionally in milk from cows nearly dry; in such cases rancidity develops 12 to 20 hours after milking.

(2) *Phosphatase.*

This enzyme attacks organic phosphorus compounds in milk. It is completely destroyed in 30 minutes at the temperature of pasteurisation (140 -145 F) by the " Holder " process. Thus its presence in pasteurised milk indicates inefficient pasteurisation. The " Phosphatase Test " for efficiency of pasteurisation is based on this destruction.

(3) *Proteases.*

These break down proteins and are associated with the ripening of cheese; during this process they convert part of the paracasein into more digestible forms.

(4) *Reductases.*

These are capable of " reducing " chemical compounds, that is, of removing oxygen from them. They can be tested for in milk by adding dyes which gradually lose their colour on reduction. This fact is made use of in testing for bacterial contamination as reductases produced by bacteria reduce added dyes. Slow reduction takes place even in sterile milk due to naturally occurring reductases.

(5) *Enzymes Acting on Hydrogen Peroxide.*

A number of naturally occurring milk enzymes liberate oxygen from hydrogen peroxide in conformity with the following equation :—

$$\underset{\text{(hydrogen peroxide)}}{2\ H_2O_2} = \underset{\text{(water)}}{2\ H_2O} + \underset{\text{(oxygen)}}{O_2}$$

One of them (*Catalase*) is very abundant in milk from cows affected with mastitis and this forms the basis of the " Catalase Test " for mastitis milk. Various rapid tests for determining whether milk has previously been heated depend on the absence of these enzymes in heated milk.

Enzymes Produced by Milk Bacteria.

(1) *Proteases.*

Numerous so-called " proteolytic " bacteria produce enzymes capable of breaking down the milk proteins. This change results in putrefaction but these organisms are normally held in check by the lactic acid bacteria. Putrefaction before souring may occur in conditions where the lactic acid organisms are absent or inactive as in very clean milk or pasteurised milk but the change is slow in comparison with normal souring.

(2) *Reductases.*

These are produced by many milk bacteria and the loss of colour of added dyes forms the basis of the " Methylene Blue " and " Resazurin " tests for bacterial contamination. The former test is used for milk on which bonus is paid under the Accredited Scheme and the latter for all milk under the National Milk Testing and Advisory Scheme.

SECTION II.—PROCESSED MILKS.

PASTEURISATION.

Pasteurisation was first used in Germany and Denmark shortly after 1880. It is now employed extensively for market milk and in the manufacture of butter and ice-cream and is spreading to the cheese industry.

The objects of pasteurisation are to destroy disease-producing (pathogenic) bacteria such as those causing tuberculosis, typhoid fever, undulant fever, etc., and to improve the keeping quality by checking the multiplication and growth of non-pathogenic organisms. *Fig.* 12 gives the temperatures at which the chief pathogenic bacteria which may be carried in milk are destroyed. Pasteurisation does not destroy all species of bacteria in milk. Species which form spores survive. Two non-sporing types also resist pasteurisation. These are the " thermophylic " bacteria which can grow and multiply at the temperature of " holder " pasteurisation and " thermoduric " organisms which survive but do not multiply at this temperature. Thermophylic and thermoduric bacteria are usually derived from the milking utensils and cause considerable trouble in pasteurising plants. All the pathogenic organisms and most of the souring organisms (lactic acid bacteria) are, however, destroyed.

TEMPERATURES AND TIMES NEEDED TO DESTROY THE PRINCIPAL PATHOGENIC BACTERIA WHICH MAY BE PRESENT IN MILK.

A. Organisms which may be Present in the Milk when Drawn from the Cow.

Bacillus tuberculosis (causes " tuberculosis " in man and animals)	140°F for 20 minutes
Brucella abortus (causes " contagious abortion " in cattle and " undulant fever " in humans) ...	140°F for 15 minutes
Haemolytic streptococci (cause " mastitis " in dairy cows and " septic throats " in humans)...	130°-145°F for 5 minutes

B. Organisms which may be Introduced by Persons Handling the Milk.

Bacillus typhosus (causes " typhoid fever ") ...	140°F for 5 minutes
Bacillus paratyphosus (causes " paratyphoid fever ")	140°F for 5 minutes
Bacillus enteriditis (cause of " food poisoning ") ...	140°F for 5 minutes
Bacillus dysenteriae (cause of " dysentery ") ...	140°F for 10 minutes
Bacillus diphtheriae (cause of " diphtheria ") ...	140°F for 3 minutes

Note that all the above are destroyed by " holder " pasteurisation (140°-145°F for 30 minutes).

Fig. 10.

Besides its use in avoiding the spread of disease by milk and in delaying souring, pasteurisation also ensures that the "ripening" processes in cream for butter-making and in cheese can proceed without interference by undesirable micro-organisms which would otherwise cause taints. Rapid cooling after pasteurisation to prevent the multiplication of surviving bacteria and care to avoid re-contamination are essential.

Methods of Pasteurisation.

(*a*) "*Holder*" *Pasteurisation.*

Milk is heated to between 140° and 145° F and held at this temperature for 30 minutes. It is then cooled and bottled. In some cases pasteurisation is carried out in the bottles. It is essential that the whole of the milk should reach the required temperature. Automatically recording thermometers are used in pasteurising plants, but there remains a risk that small portions of the milk may not reach the necessary temperature. It is preferable to test the milk for efficiency of pasteurisation rather than to rely on temperature records. The "phosphatase test" is now available for this purpose. Milk cannot be sold under the label "pasteurised" unless the plant has been officially approved. Until recent years only "holder" type plants could in Britain receive this approval. The "holding" system of pasteurisation has the advantage of not seriously reducing the volume of cream (the *cream-line*). Temperatures much above 145° F reduce the cream-line and give the appearance of a smaller fat percentage although the latter is actually not affected.

(*b*) "*Flash*" *Pasteurisation.*

In this type milk is quickly heated to about 160° F, maintained at this temperature for a few seconds and then quickly cooled. Heating is carried out in a variety of ways in different plants. Examples are "Biorisation" in which the milk is passed into the heating chamber in a fine spray, the "High-Temperature Short-Time" (H.T.S.T.) continuous flow method and "Stassanisation" in which the milk is heated in thin layers. The two last-named are improvements on the original "flash" method and provide a more effective control of temperature with consequently more complete destruction of pathogenic organisms and less reduction of nutritive value. Moreover the cream-line is not so seriously affected.

Effect of Pasteurisation on Milk Constituents.

(*a*) "*Holder*" *Pasteurisation.*

The three main constituents, fat, casein and lactose, are not affected. Up to 5 per cent of the lactalbumin may be clotted but no appreciable effect on the feeding value is thereby caused. A small proportion of the soluble calcium and phosphates is rendered insoluble. The only vitamin seriously affected is Vitamin C. The destruction of this vitamin is less if the milk is heated out of contact with air. Traces of copper increase this destruction. Milk is not, however, a rich source of this vitamin. Large scale feeding experiments

with school children have failed to reveal any appreciable difference in nutritive value between raw and " holder " pasteurised milk.

(*b*) *" Flash " Pasteurisation.*

The original method of flash pasteurisation often imparted a " cooked " taste and seriously impaired the nutritive value. The H.T.S.T. process and Stassanisation much reduce this loss in feeding value. Exact comparisons as regards effect on nutritive value between these two processes and holder pasteurisation are not yet available.

HOMOGENISATION.

The object of homogenisation is to reduce the fat globules to tiny dimensions so small that they do not rise to the surface. The separation of cream is thus prevented. This is essential for some manufacturing processes. The first " homogeniser " was used in France in 1899. There are now three distinct types :—(1) High Pressure Type; (2) Low Pressure—Rotary Type; (3) The Sonic Vibrator. *High pressure types* force the milk under high pressure through a very small orifice to break up the fat globules. The pressure can be varied by the operator. *Low pressure-rotary types* use pressures below 500 lb. per sq. in. and the fat globules are broken up by a shearing action. Probably they do not produce quite such small globules as the high pressure types. *Sonic Vibrators* (or Oscillators) use sound waves of high frequency to break up the fat globules. They are recently introduced and not yet so commonly used as the other two types.

Homogenisation reduces the size of the fat globules below 0.0002 mm. Cream does not rise nor can butter be produced by churning. It also increases the viscosity of milk. This latter effect is probably due to the much larger total surface area of the smaller globules causing more protein to be absorbed on their surfaces. On clotting with rennet homogenised milk produces a softer curd than does the untreated milk. Homogenised milk becomes rancid more quickly on account of the greater surface area of fat exposed to lipases (fat splitting enzymes). Homogenisation is used for the following purposes :—

(*a*) In serving liquid milk in restaurants it is an advantage if cream does not rise, as this avoids the necessity of shaking each time milk is poured out.

(*b*) Cream for table use is frequently homogenised to produce a more uniform and more viscous product. The increase in viscosity imparts a " rich " appearance.

(*c*) In sweetened condensed milk the added sugar increases the viscosity so that the product does not " churn " in the tins if these are subjected to excessive shaking. In the unsweetened type (evaporated milk) homogenisation is used to increase the viscosity and so achieve the same result.

(*d*) In making ice-cream the whole mixture is passed through the homogeniser after pasteurisation. This procedure gives a smoother texture and the mixture can be frozen without risk of churning in the freezer.

(*e*) In making cream-cheese homogenisation ensures even distribution of the fat through the cheese.

(*f*) For the production of bottled " sterilised " milk it is customary to homogenise before sterilising. Otherwise plugs of cream form in the bottle necks.

(*g*) Reconstituted milk or cream may be made in the homogeniser by mixing butter with skimmed milk.

" Emulsifiers " are similar to homogenisers in effect but do not reduce the size of fat globules so markedly. They are used in making " coffee cream."

STERILISED MILK.

Sterilisation destroys bacteria and other micro-organisms more completely than does pasteurisation as the milk is heated to approximately 212 F. The keeping quality is thereby superior and sterilised milk will keep for some weeks, but this advantage is gained at the expense of a considerable reduction in nutritive value. Some of the vitamins are destroyed and the bone-forming minerals are rendered less easy of assimilation.

The milk is first filtered or clarified, pre-heated and then passed through the homogeniser. Following homogenisation the milk is filled into hot sterilised bottles with narrow necks and is sterilised in the bottles.

Sterilisation is carried out in steam-jacketed tanks or steam chests and may consist of maintaining a temperature of 212 F for 25 minutes or of gradually raising the temperature to 222 F by means of steam under pressure.

IRRADIATED MILK.

Milk is sometimes subjected to ultra-violet rays from a mercury vapour or carbon-arc lamp in order to increase the Vitamin D content and thus provide protection against rickets in children. Too long exposure results in taints due to fat oxidation; the distance of the milk from the lamp and time of treatment must be carefully controlled. Either raw or pasteurised milk may be treated but as low a temperature as possible during treatment should be maintained. Subsequent pasteurisation or storage does not result in loss of Vitamin D. Irradiation also destroys bacteria; a reduction of up to 98.5 per cent in the total number of micro-organisms is obtained. The cream-line is unaffected and the taste unaltered.

Other methods of increasing the Vitamin D content of milk are the addition of Vitamin D concentrates and the feeding of irradiated yeast to cows

FERMENTED MILKS.

YOGHOURT.

This sour milk drink is made in the Near East, particularly in Turkey, Bulgaria, Armenia and Egypt. In some regions sheeps' or buffalos' milk is used. It is now made in Canada and the U.S.A. entirely from cows' milk.

The milk is first concentrated to about two-thirds of its volume in a condensing pan. Alternatively 5% of dried skim milk may be added to increase the proportion of total solids. Following condensation it is subjected to heat treatment to destroy micro-organisms. It may be heated to 212°F and immediately cooled to 112°F or maintained for 30 minutes at 180°F. "Flash" pasteurisation may be employed as an additional alternative.

After cooling to 112°F a culture of "Yoghourt" organisms is added. Yoghourt cultures contain three species of micro-organisms, viz.:—*Lactobacillus bulgaricus*, *Plocama-bacterium yoghourtii* and *Streptococcus thermophilus*. The two first-named organisms produce lactic acid while the third is responsible for the aromatic flavour of the product. After adding 2% of the culture and mixing thoroughly the milk is placed in quarter-pint bottles and incubated at 112°-115°F for 3 hours. Coagulation takes place during incubation and the bottles are finally placed in a refrigerator for 8 hours. Yoghourt should keep from 4 to 8 days.

Kefir.

This product originated in the Caucasus Mountains but is now also made in the U.S.A. Unlike yoghourt kefir contains alcohol as well as lactic acid; average samples contain 0.9% lactic acid and 1.1% alcohol. Kefir "grains" used to bring about the fermentation contain casein, yeasts and bacteria.

SECTION III.—MILK PRODUCTS.

CREAM.

Cream is used for table purposes, for the manufacture of ice-cream and for churning into butter. Table cream usually has about 20-25% fat, but "whipping cream" may have more. For butter-making the fat content is usually above 25%. Although butter has been made from time immemorial it was formerly made by churning milk. The use of cream for churning was a comparatively late development. Cream raising in pans was, however, employed in the eighteenth century. It is at present illegal to sell cream and the so-called "cream" in ices and buns is a product made from milk powder, margarine, sugar and gelatine. When the sale of cream is again permitted it is very desirable that a minimum percentage of milk fat should be laid down.

Rise of Cream.

The specific gravities of milk fat and of skimmed milk are 0.92-0.94 and 1.036 respectively. The velocity of rise of individual fat globules can be calculated from Stokes' Law as follows :—

$$v = \frac{2r^2 (d - d^2)}{9N}$$

, where v is the velocity of rise
r is the radius of the globule
d is the specific gravity of the skimmed milk
d^2 is the specific gravity of the fat globule
N is the relative viscosity of the skimmed milk.

Thus bigger globules rise quicker than small ones and the rise can be accelerated by increasing the difference in specific gravity between the fat and skimmed milk or by reducing the viscosity of the latter.

Cream rises quicker than can be accounted for by the above equation. This is due to the collecting together of the globules into " clumps " which behave like very large globules. Addition of materials such as gelatine or gums increases the size of the clumps and accelerates creaming. Heating milk above 145 F breaks up the clumps and makes creaming much slower. Thus pasteurisation delays creaming. At low temperatures clumps form more readily and the rise of cream is accelerated.

The clumps are not pure fat. They contain also skimmed milk between the fat globules. The densest clumps have only about 50 per cent of fat. The more closely the globules pack together in the clumps the higher the fat content of the cream but the thinner the cream layer The net result of these factors can be expressed as follows :—

At low temperatures clumps form readily causing quick creaming, but the fat globules are loosely packed in the clumps. This results in a thick cream layer but a low fat percentage in the cream. At high temperatures clumps do not form so readily so that creaming is slow ; also the fat globules are more closely packed in the clumps producing a thin cream layer but a high fat percentage in the cream.

Mechanical Separation of Cream.

The mechanical separator first made in 1879 substitutes " centrifugal force " for the force of gravity. At normal running speeds the force exerted on the fat globules by this machine is more than 1,000 times that in cream raising in pans. Milk is usually heated to a temperature of 84 -95 F before separation partly because its viscosity is lower at this temperature and partly on account of the greater difference in specific gravity between milk fat and separated milk as compared with ordinary temperatures. The latter fact is due to the fat expanding with rise in temperature more quickly than separated milk. Globules less than 0.001 mm. in diameter are not removed by the separator.

Percentage of Fat in Cream from the Separator.

The percentage of fat in cream is controlled by :—

(*a*) *The setting of the cream screw.* This varies the amount of skimmed milk which is discharged with the cream and hence the percentage of fat in the latter.

(*b*) *The speed of the machine.* Centrifugal force is proportional to the square of the velocity. For this reason high speeds give cream richer in fat. The effect of speed is more pronounced when the cream screw is set to produce rich cream.

(*c*) *The temperature of the milk.* Richer cream is produced at low than at high temperatures. This is due to the slower flow of cream through the cream opening at the lower temperatures which permits less of the skimmed milk to accompany it. An exception is that at very low temperatures a decrease in fat content may occur through adherence of the cream to the inside of the bowl. This also results in a high loss of fat in the skimmed milk.

(*d*) *The percentage of fat in the milk.* Under similar conditions the cream represents the same proportion of the original milk irrespective of the fat content of the latter. Thus milk of high fat content will produce richer cream than poorer milk.

(*e*) *Rate of inflow of milk into the separator.* Reducing the rate of inflow increases the percentage of fat in the cream. This is because a reduction in the rate of inflow leads to an increase in the proportion of milk discharged through the skimmed milk outlet.

(*f*) *Amount of Flushing.* If the machine is flushed with water or skimmed milk just after the milk has passed through in order to remove cream remaining in the machine, this will dilute the cream and reduce the percentage of fat.

(*g*) *Formation of Separator Slime.* When power separators are run continuously for 4 to 5 hours the layer of slime formed inside the machine reduces the outflow from the skimmed milk outlet and diverts more skimmed milk through the cream outlet thus reducing the percentage of fat in the cream.

A mechanical separator should remove 99 per cent of the fat from milk. With efficient operation the separated milk should not contain more than 0.01 per cent of fat. Cream raising in shallow pans removes only about 80 per cent of the fat and in deep pans about 90 per cent.

BUTTER-MAKING.

Hindu writings indicate that butter was made before the year 2000 B.C. and there are references to it in the oldest parts of the Bible. The Greeks and Romans used butter more as an ointment with medicinal properties than as a food. It was exported from Scandinavia in the twelfth century.

Butter-making involves *phase inversion.* In cream fat globules are present dispersed in the milk serum. Thus the fat is here the *dispersed* phase and the milk serum the *continuous* phase. In butter the fat is continuous and droplets of skimmed or separated milk are dispersed in it. This interchange of positions between the two constituents of an emulsion is termed phase inversion. Inversion of an emulsion of an oil in water can readily be effected by chemical means,

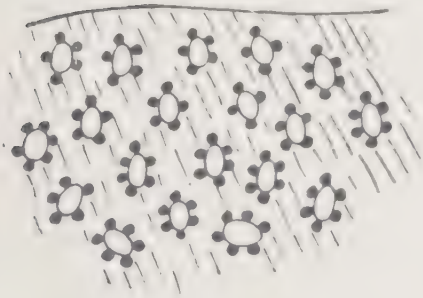
but in butter churning it is brought about mechanically. The precise way in which this happens is not clear. The two most plausible alternative explanations are stated in a simplified form below.

The "Foam" Theory.

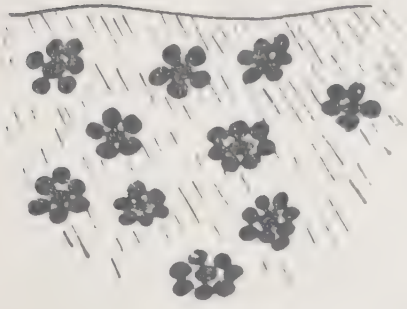
The churning of cream in an incompletely filled butter churn creates a foam as minute bubbles of air become included. The fat globules gradually collect round the edges of these air bubbles where air and liquid meet. Clumps of fat globules form and gradually increase in size. In time they grow to such a size that the foam suddenly collapses leaving grains of butter. The sequence of events is illustrated diagrammatically in *Fig.* 11.

SEPARATION OF BUTTER GRAINS DURING CHURNING AS EXPLAINED BY THE "FOAM" THEORY.

Churning produces a foam with air bubbles dispersed in the cream.



Fat globules collect in "clumps" round the edges of the air bubbles.



The "clumps" increase in size until the foam suddenly collapses leaving the butter "grains" suspended in the buttermilk.

Fig. 11.

This theory explains several difficulties in butter-making. Filling the churn too full—which is well known to retard butter formation—restricts foam formation through lack of air. The fact that churning above 93°F will not produce butter is explained by the failure of the fat globules to form clumps above this temperature. Churning at very low temperatures is also ineffective because the fat globules become solid and will not adhere together to form clumps.

The Phase Inversion Theory.

According to this theory the fat globules in cream are prevented from coalescing by a layer of " hydrated protein " (protein containing water) round each globule. Churning breaks these layers enabling the fat globules to coalesce. This theory is supported by the fact that butter will not form in the absence of traces of protein.

Ease of Churning.

This depends on :—(1) The size of the fat globules; large globules churn most readily. (2) The acidity of the cream; the lactic acid produced during cream ripening decreases the viscosity of the cream and facilitates churning. Too high acidity, however, will precipitate casein in white specks which are difficult to remove from the butter. (3) The temperature of cream ripening.

The Flavour of Butter.

In order to obtain a desirable flavour in butter the cream should be pasteurised to destroy unwanted types of bacteria and should then be ripened by pure cultures of the appropriate strains of lactic acid bacteria. The chief flavouring agent produced during cream ripening is " diacetyl " which has a very powerful buttery odour and taste. Full flavoured butters contain 3 to 5 parts per million of this substance, mild flavoured butters 0.5 to 1.5 and medium flavoured 1.5 to 3 parts per million. Defects in flavours are due to splitting or oxidation of the fat as described earlier.

Composition of Butter.

This varies slightly but the following are average figures :—

	Per cent
Fat	82.5
Casein	1.5
Lactose	2.0
Mineral matter (including salt) ...	2.0
Water	12.0
	100.0

The figures for casein and lactose are often considerably less than the above and the percentage of fat higher.

The Sale of Food and Drugs Act fixes a maximum limit of 16 per cent of water in butter and preservatives may not be added.

MARGARINE.

The modern inclusion of milk products in the manufacture of margarine warrants its consideration here. Margarine was first made about 1870 from animal fats, but it is now manufactured from vegetable oils. The oils extracted from groundnuts, coconuts, palm kernels and soya beans are mainly used. The oils are decolourised, deodorised and purified until tasteless and then emulsified into separated milk which has previously been ripened with cultures of lactic acid bacteria. Oils from soya bean and groundnut are too soft and are first hardened by combination with hydrogen gas, the process being known as *hydrogenation.*

A common mixture consists of four parts of oil and one part of separated milk. During emulsification, which is carried out at 25° to 40°C according to circumstances, concentrated preparations of vitamins A and D are added as vegetable oils are lacking in these vitamins. The emulsion is then poured on to the surface of a revolving drum maintained at 15° C and is scraped off as a thin film. This is left for some hours to develop flavour and is then worked like butter. The Sale of Food and Drugs Act permits the addition of up to 10 per cent of butter. It also fixes a maximum limit of 16 per cent of water.

Unlike butter the vitamin content of margarine can be maintained at a constant level all the year round and the hardness of the fat is under complete control. Its chemical composition is very similar to that of butter, the main differences being in the proportion of the various glycerides in the fat.

THE CLOTTING OF MILK.

Clotting of milk takes place in three distinct ways.

(1) *Through Souring.*

In this case the lactic acid developed from lactose by bacterial action causes coagulation of the casein. A similar effect occurs when dilute acids are *added* to milk or separated milk. Casein is commonly prepared from separated milk by the addition of sufficient acetic acid to produce an acidity corresponding to a pH of 4.6 (the "isoelectric point" of casein).

(2) *By Heat.*

Total coagulation of fresh milk by heat at temperatures below the boiling point does not occur unless the sum of the percentages of lactalbumin and lactoglobulin exceeds 0.9 as is the case in colostrum. Normal fresh milk clots at temperatures above its boiling point; the relation between temperature and time required for coagulation is given below; (based on experiments by Holm, Deysher and Evans).

Temperature °C	*Time required for coagulation minutes*
131	10
128	12
123	20
116	45
114.5	60

Developed acidity lowers the temperature of clotting. This effect makes essential the rejection at pasteurising, sterilising, condensing and drying plants, of milk with an acidity higher than normal. It is illustrated by the following observations by McInerney on the effect of added lactic acid on the temperature of coagulation :—

Percentage acidity	*Temperature of coagulation* °F
0.25	180
0.35	150
0.40	100-110
0.50	75-80
0.57	60-65

Partial coagulation by heat occurs in the *skin* formed on the surface of heated milk and in the *milk stone* which develops in milk processing plants. The skin contains all the milk proteins with variable proportions of fat and of phosphate of lime. Milk stone forms most readily when milk foams on heating due to the gases dissolved in it. Its formation reduces the efficiency of heating plants and makes cleaning more difficult. In making condensed milk the tendency to form milk stone is reduced by *forewarming* (heating to near boiling point) before condensing; this removes dissolved gases.

(3) *By the Action of Enzymes.*

Casein is coagulated in the animal stomach. The enzymes responsible are *pepsin* in the case of an adult animal and *rennin* in the suckling animal. This coagulation is quite distinct from those brought about by acids and by heat. The chemical composition of the casein undergoes change and it is customary to recognise this fact by terming the protein in the curd " paracasein."

Clotting enzymes are present in certain plants, such as *Butterwort* and *Lady's Bedstraw*, but their use in cheese-making is not common owing to the production of bitter flavours. Some bacteria clot milk without souring through the activity of clotting enzymes produced by them.

CHEESE-MAKING.

Cheese, like butter, is mentioned in the oldest parts of the Bible. It was made in Greece as early as 1000 B.C. It was commonly used as food in the German States in 800 A.D. The monks were skilled in cheese-making and by 100 A.D. there was an important trade in cheese.

Rennet.

Rennet is prepared from the fourth stomach of the calf. Commercial rennet preparations contain small amounts of pepsin as well as rennin. Rennin acts in much less acid conditions than pepsin, the optimum (best) degrees of acidity for the activity of these two enzymes corresponding to pH 5.4 for rennin and pH 2.0 for pepsin. The presence of large amounts of the latter enzyme is undesirable, as it leads to bitterness in cheese. Purification of rennets from pepsin, has been achieved in recent years. Purified rennets can now be purchased

in tablet as well as in liquid form; tablets retain their activity better. The activity of these preparations can be checked by noting the time needed to coagulate milk under rigidly standardised conditions.

THE ACTION OF RENNET ON MILK.

This consists of two stages, the first being the attack of rennin on the casein and the second the coagulation of the main products of this attack. The latter stage does not occur at temperatures below 10°-20° C. Casein is converted into paracasein and a proteose (a soluble nitrogen compound) is set free and passes into the whey. Rennin is most active at 41° C and is one of the most powerful enzymes known. Very pure preparations of it have been made of which 1 part can coagulate 2,310,000 parts of milk. Commercial rennets are only very dilute solutions of rennin.

RATE OF CLOTTING.

This depends on a number of factors of which the following are the chief :—

(1) *Concentration of Rennet.*

The time of coagulation is usually inversely proportional to the concentration of rennin. Dilution of milk with water also has a retarding effect, but up to 10 per cent of water may be added before this is appreciable.

(2) *Temperature.*

The following results due to Lind illustrate the effect of this factor. Above 43° C the enzyme is gradually rendered less active.

Temperature °C.	*Time of Coagulation Minutes*
23	36
28	11.1
33	7.5
38	6.5
43	6.0
48	6.6

Clotting is quickest at a temperature of 40° to 41° C.

(3) *Acidity of the Milk.*

Development of lactic acid through the addition of *starter* in cheese-making hastens the coagulation. The following figures due to Davis illustrate this effect.

Percentage of lactic acid developed	*Time of coagulation Minutes*
Nil	22.5
0.01	12.0
0.025	5.25
0.05	2.33

Both stages of rennet action appear to be accelerated by small increases of acidity. Alkalies check rennet action; there is no clotting at pH 7.53. This may be one explanation of the slow working of cheese made from the milk of cows affected with mastitis. Such milk is commonly slightly alkaline.

(4) *The Mineral Matter of the Milk.*

Calcium in the form of soluble compounds is essential to coagulation. Its effect is mainly on the second stage of rennet action. Milk which has been heated clots more slowly, as heat renders some of the calcium insoluble. The addition of a soluble calcium compound restores normal clotting properties. The effect of calcium is, however, complicated by the proportions of the other mineral elements and the time of coagulation cannot be calculated from the percentage of soluble calcium alone.

(5) *Other Factors.*

Storage of milk for some hours slightly increases the time needed for clotting. This explains the slightly longer coagulation time observed with evening than with morning milk. High proportions of lactalbumin and lactoglobulin retard coagulation; this fact has been advanced as another cause of the slow clotting of mastitis milk.

THE RENNET CURD.

The curd obtained with rennet contains more mineral matter than that produced by acid coagulation and is much more plastic. Most of the fat is mechanically " entangled " in the curd, whereas most of the lactose, lactalbumin and lactoglobulin and a large proportion of the mineral matter remain in the whey. The carotene and vitamins A and D are found in the curd. The water-soluble vitamins (B and C) go into the whey.

The curd is elastic and shrinks as the percentage of lactic acid rises during the " scalding " and " piling " processes in cheese-making. The rise of temperature during " scalding " also shrinks the curd and allows the whey to escape. The curd consists of a network of fibres of a complex compound of " calcium paracaseinate " and " tricalcium phosphate." The fat globules are entangled in this network. At cutting, the curd contains about 87 per cent of moisture which is reduced to about 40 per cent when the curd is milled. The process is controlled by the determination of total acidity at each stage. Standard figures for these acidities depend on the type of cheese. *Fig.* 12 shows the average percentages of total acidity at each stage in the making of Cheddar Cheese, which is produced in larger quantities than any other type.

ACIDITIES AT VARIOUS STAGES IN THE MANUFACTURE OF CHEDDAR CHEESE—(Figures from E. R. Ling).

(The acidities are expressed as percentages of lactic acid although this is present only in the " developed " acidity).

	Percentage Lactic Acid
Milk on addition of rennet	0.19 — 0.23
Whey after cutting the curd	0.14 — 0.15
Whey at " pitching "	0.18 — 0.19
Whey from " piled " curd	0.24 — 0.28
Whey expressed from curd before milling	0.65 — 0.95
Whey expelled from cheese while in the press	0.95 — 1.10

Note the reduction in acidity during coagulation. This is due to the removal of casein from the whey into the curd. Casein contributes a large proportion of the " natural acidity " of milk.

Fig. 12.

From 1 to 3 per cent of salt is usually added to the curd after milling. This has a number of desirable effects. It increases the solubility of the protein, checks the action of bacteria and enzymes and assists in the draining of the whey in the press. Too much salt will retard cheese ripening.

Soft and Hard Curds.

Milk which produces soft curds is suited to infant feeding but higher losses of fat and casein occur during cheese-making. Clotting with rennet is slower in soft curd milk and the curd ripens too quickly and has an inferior flavour. The hardness of the curd depends on the following factors :—

(1) *The Individual Cow.*

Attempts have been made to reserve *soft curd* milk for infant feeding, but the number of cows producing it is too small. They occur most frequently in Friesian and Ayrshire herds. Milk low in solids generally forms a soft curd.

(2) *Udder Disease.*

Milk from cows suffering from mastitis produces milk slow in coagulation and gives a very loose curd.

(3) *Temperature of Clotting.*

Clotting at low temperatures produce a softer curd than at higher temperatures.

(4) *Acidity at Renneting.*

The last two factors control the methods used in the manufacture of the various types of cheese. They can be varied to produce different types.

The hardness of the curd (*curd tension*) is reduced by diluting the milk with water, pre-heating, homogenising and additions of lime water, but the effects are small.

Milk which has been heated to high temperatures forms a softer curd. It is partly for this reason that evaporated milk is successful in infant feeding. Passing milk through a "permutit" filter—such as is used in water softening plants—greatly reduces curd tension by replacing calcium by sodium, but this has ill-effects on nutritive value. Addition of *Calgon* (sodium hexametaphosphate), also used in detergents, produces soft curds but effects on nutritive value are not yet sufficiently understood to enable the process to be recommended. Homogenisation and heat treatment are the safest and most practicable methods of reducing "curd tension." In order to produce a softer and more easily digested curd milk for use in calf feeding is diluted with water.

Cheese Ripening.

The changes during ripening can be grouped as follows :—

(1) *Loss of Weight through Evaporation of Water.*

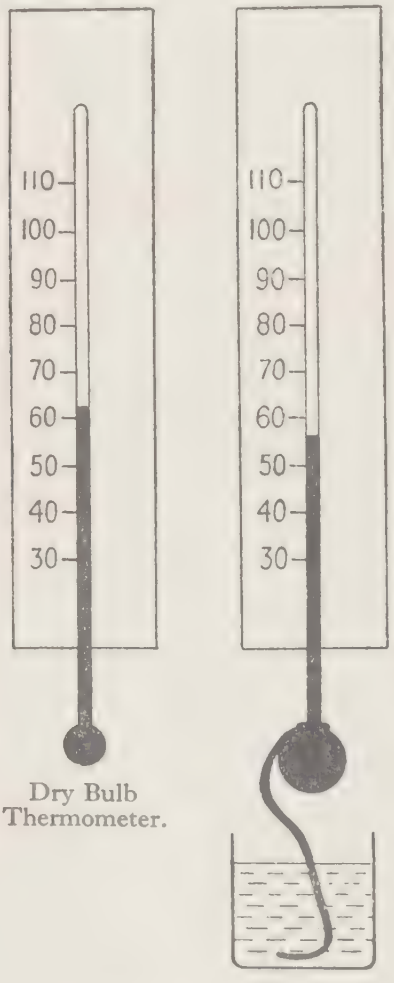
Coating the cheese with paraffin wax reduces this loss but is rarely carried out. The cheese-maker normally relies on controlling the humidity of the ripening room. The rate of evaporation of water will depend on the *relative humidity* of the room, that is, the water vapour in the air expressed as a percentage of the quantity of water vapour which would be present if the air were completely saturated.

Thus the relative humidity of the air

$$= \frac{\text{Water vapour present}}{\text{Water vapour held by saturated air at the same temperature}} \times 100$$

It can be determined by means of " wet and dry bulb " thermometers as is explained in *Fig.* 13.

DETERMINATION OF " RELATIVE HUMIDITY " OF THE AIR BY MEANS OF WET AND DRY BULB THERMOMETERS.



Dry Bulb Thermometer.

Wet Bulb Thermometer.

The bulb of the " wet bulb " thermometer is kept moist by wrapping with damp muslin maintained in a wet condition by means of a cotton thread which dips into a small jar of distilled water (or rain water).

Provided that the atmosphere is not completely saturated with water vapour, the wet bulb thermometer will record a lower temperature than the other thermometer owing to the heat abstracted from its bulb through the evaporation of water. The lower the relative humidity of the atmosphere the greater will be the difference in temperature between the two thermometers. Tables are provided with the instrument by means of which the relative humidity of the atmosphere can be calculated from the difference in temperature between the thermometers.

It is essential to read the thermometers to the nearest one-tenth of a degree.

Fig. 13.

If the air is kept completely saturated with water vapour by swilling the floors with water and hanging damp cloths in the room, no loss by evaporation occurs, and there may be some slight gain in weight by condensation of water on the cheese. A completely saturated atmosphere is, however, undesirable as it favours mould growth. It is usually recommended that in the early stages of ripening the atmosphere of the room should have a relative humidity of 85-90 per cent and that this should be increased to 90-95 per cent for prolonged storage. The humidity can be controlled by installing an air conditioning plant. If this costly equipment is not available, humidity can be increased as desired by wetting the floors, or reduced by opening windows and improving ventilation. Surface mould growth can be checked by painting the cheese with a 5 per cent solution of propionic acid or of calcium propionate.

(2) *Formation of Lactic and other Acids.*

Bacterial action on the small percentage of lactose remaining in the curd continues during ripening. The lactose is completely converted into lactic and other acids.

(3) *Digestion of Proteins with the Formation of Soluble Nitrogenous Compounds.*

From the nutritive aspect this is much the most important change and can be compared to protein digestion in the alimentary canal of an animal. Enzymes convert a large proportion of the protein into compounds soluble in water. The process is more complete in soft than in hard cheeses. The former have more than two-thirds of their nitrogen in soluble form after ripening as compared with about one-third in the latter. This digestion of proteins is slower at low temperatures and is also retarded by increasing the proportion of salt added to the curd. Loss of moisture retards protein digestion; small cheeses tend to ripen more slowly than large ones owing to their quicker loss of moisture. Ripening enzymes are supplied by rennet; increasing the quantity of rennet accelerates ripening. Other sources of *proteolytic* (protein-splitting) enzymes are bacteria and moulds. The latter play a predominant part in ripening soft cheeses; with hard cheeses, bacteria are the more important agents.

(4) *Development of Flavour.*

The substances responsible for the flavour of cheese have not been identified. There is evidence that flavouring agents are produced from the fatty acids set free from the fat by the action of lipases. The substances probably responsible are *esters*, that is, compounds of fatty acids with alcohols. In cheese with marked mould growth, such as Stilton, Wensleydale or Roquefort, lipases are probably provided by the moulds. The addition of 0.025 gram of lipase per gallon of milk produces a cheese with a very strong flavour. Cheese made from pasteurised milk is very slow in developing flavour probably because pasteurisation reduces the quantity of lipases in milk.

Cheese from Pasteurised and "Reconstituted" Milk.

Most Dominion cheese, such as Canadian or New Zealand, is made from pasteurised milk which is slower in *working* and needs a

higher acidity at renneting. The whey is more acid and dissolves more calcium and phosphorus from the curd leaving it more *rubbery*. Spray-dried milk may be *reconstituted* by adding the correct amount of water and then used for cheese-making. The cheese is similar to that from pasteurised milk.

Processed or Crustless Cheese.

This is made from ripened cheese. It is marketed in tinfoil which prevents loss of moisture and enables the product to keep indefinitely. After removing the bandages and crusts the cheeses are sliced and slices of different types of cheese blended and ground. The material is heated in a steam-jacketed vat to about 140°-150°F with constant stirring. To avoid fat separation on cooling, emulsifying agents are added of which the most suitable is sodium citrate. Rochelle salt (sodium potassium tartrate) and disodium phosphate are sometimes used for this purpose, but the latter tends to blacken tinfoil if used in concentrations exceeding 2 per cent.

Composition of Cheese.

This varies considerably and there are no minimum standards prescribed by law. It is an offence to sell cheese containing fats other than milk fat except where this is indicated on the label. The abstraction of fat from the milk used, or the addition of separated milk, will reduce the fat to less than 45 per cent of the dried cheese and also result in an abnormally low ratio of fat to milk proteins, but the vendor of such cheese is not compelled to declare the deficiency in fat. *Fig.* 14 gives the average composition of the best known makes of cheese.

COMPOSITION OF VARIOUS MAKES OF CHEESE.

(Figures due to Nicholls).

Make of Cheese	*Percentage water*	*Percentage fat*	*Percentage of other substances (chiefly proteins with some mineral matter and lactic acid)*	*Percentage fat in the dried cheese*
Cheddar ...	36.7	31.8	31.5	50.2
Cheshire ...	40.5	30.0	29.5	50.3
Caerphilly ...	44.7	28.4	26.9	51.2
Derby ...	40.0	30.6	29.4	50.7
Lancashire ...	44.3	27.4	28.3	49.1
Leicester ...	39.8	30.2	30.0	50.0
Blue Stilton ...	37.6	35.7	26.7	57.2
White Stilton ...	41.8	32.4	25.8	55.4
Wensleydale ...	45.0	27.7	27.3	50.2

Note that the percentage of fat in the dried cheese is the most constant figure. If this is less than 45% abstraction of fat or addition of separated or skimmed milk is indicated.

Fig. 14.

ANNATTO.

This reddish-yellow colouring matter used in cheese and butter-making is prepared from the seeds of the shrub *Bixa orellana*. It contains the pigment *bixin* which combines with the proteins in cheese. In the preparation of butter annatto, bixin is dissolved in oil. It passes into the butter-fat during butter-making.

CONDENSED MILK.

The sweetened type was first made commercially in 1856 and the unsweetened in 1883. The former is now usually referred to as *condensed milk* and the latter as *evaporated milk*. The Food and Drugs Act lays down the following minimum standards of composition.

	Percentage Fat	*Percentage Total Milk Solids*
Condensed whole milk (sweetened and unsweetened) ...	9	31
Condensed skimmed milk (sweetened)	Nil	26
Condensed skimmed milk (unsweetened)	Nil	20

Containers must be clearly labelled to indicate whether made from whole or skimmed milk and whether sweetened or not. If made from skimmed milk the statement " Not to be used for babies " must also appear on the label.

It is apparent from the above figures that milk must be condensed to about 40 per cent of the original bulk and that the ratio of fat to total solids should be 9 : 31. Milk is therefore usually standardised to this ratio before condensing by the addition of the necessary quantity of cream (if deficient in fat) or of separated milk (if high in fat).

Methods of Manufacture.

A. CONDENSED MILK (Sweetened).

(i) *Preparation of the Milk.*

The incoming milk is examined for flavour and samples are taken for :—

(*a*) *Determination of percentages of fat and of non-fatty solids.* On the basis of the results the milk is standardised to the above ratio of fat to total solids.

(*b*) *Estimation of acidity and sediment.* Milk with a total acidity of over 0.19% (expressed as lactic acid) is usually rejected to minimise the danger of clotting on heating.

(*c*) *Estimation of bacterial contamination.* Tests include the *Methylene Blue* and *Resazurin* tests, examination for *coliform* bacteria, and the *fermentation test* for gas-producing organisms.

(*d*) *Determination of " heat stability."* The object of these tests is to determine whether clotting may occur on heating. Milk which clots on the addition of a 68 per cent solution of alcohol in water is not " heat stable." Milk of low heat stability

will also clot when heated with a dilute solution of potassium phosphate.

The milk is then filtered or clarified to remove slime, cooled to 60°F to retard the growth of bacteria and " standardised " as previously explained.

(ii) " *Forewarming* " *and addition of sugar.*

The milk is now heated to near its boiling point. This forewarming removes gases and thus reduces the tendency to " foam " with consequent formation of " milk stone." It destroys milk enzymes, moulds and also many bacteria. It ensures the rapid dissolving of the sugar subsequently added. Moreover, it increases the *heat stability* and reduces the tendency of the condensed milk to thicken with age. after forewarming, fifteen to sixteen pounds of sugar are added per 10 gallons of milk.

(iii) *Evaporation of water.*

Water is evaporated by heating the milk in " vacuum pans " under reduced air pressure. Sufficient vacuum is maintained to cause the milk to boil at 130-145°F. Much less reduction of nutritive value occurs than would be the case if milk were boiled under normal atmospheric pressures at about 212°F. As water is evaporated the specific gravity of the milk rises. The correct stage at which to stop the process is judged by examination of the condensed milk; its specific gravity is determined by means of a hydrometer. The product is then cooled rapidly. Too slow cooling causes thickening and a gritty texture due to the formation of minute crystals of lactose. Containers are sometimes filled under a vacuum to ensure absence of air which would cause deterioration. Cans must be labelled with the number of pints of milk to which the contents are equivalent.

Sweetened condensed milk is not sterile but the high percentage of sugar prevents bacterial growth, as it also does in jams.

B.—Evaporated Milk (Unsweetened).

In the manufacture of this product, preparation of the milk, forewarming and the evaporation of water under reduced pressure are carried out as previously described for condensed milk. After condensation the product is usually homogenised. This causes the casein and lactalbumin to adhere to the minute fat globules produced by homogenisation and gives a smoother product. A gritty texture does not occur in evaporated milk as, owing to the higher percentage of water, lactose crystals do not form.

As no sugar is added, evaporated milk must be sterilised to prevent bacterial growth. For this purpose the cans after filling are heated to 240°F under pressure. The temperature of sterilisation must be rigidly controlled to avoid thickening. An abnormal composition of the mineral matter in the milk *ash* will also cause thickening during sterilisation and it may be necessary to correct the mineral balance. Containers must be cooled rapidly after sterilisation to avoid bulging of the ends. As with condensed milk the equivalent number of pints of whole milk must appear on the labels.

Typical analyses of condensed and evaporated milk and of condensed separated milk are given in *Fig.* 15.

COMPOSITION OF CONDENSED AND EVAPORATED MILK.
(Figures due to Cox).

	Condensed whole milk		*Condensed separated milk*
	Sweetened	*Unsweetened*	*Sweetened*
	Percentage	*Percentage*	*Percentage*
Water	25.1	67.5	27.0
Fat	9.6	9.1	0.3
Lactose	13.1	12.7	14.3
Sucrose (Cane Sugar)	41.9	Nil	46.6
Proteins	8.4	8.8	9.5
Mineral Matter ...	1.9	1.9	2.3

Fig. 15.

MILK POWDERS.

The main difficulty to be overcome in drying milk is the avoidance of loss of solubility in the resulting powder due to chemical changes in the milk proteins. To counteract this tendency, bicarbonate of soda and sugar were formerly added during drying. Modern milk powders made without these additions date from 1890. Processes are designed to shorten the time of heating and thus avoid loss of solubility.

Legal standards exist only for fat content. The Public Health (Dried Milk) Regulations of 1923 impose the following standards :—

	Percentage of Fat.
Dried, full-cream milk ...	Not less than 26
Dried, partly-skimmed milk ..	Not less than 8, but less than 26
Dried, skimmed milk	Less than 8.

There are two main types of milk drying plants in operation, *roller* driers and *spray* driers.

Roller Drying.

Milk is allowed to drip on to rotating metal rollers, heated internally by hot water or steam, and dries as a thin film. Scrapers remove this film which is then ground to a powder. Some roller driers work at atmospheric and others at reduced pressures. The latter produce a more soluble product as the temperature of drying is about 104°F as compared with 212°F for the former.

Spray Drying.

In spray-drying plants, the milk is usually first condensed in vacuum pans until it contains about 40 per cent of total solids. In some plants homogenisation precedes condensation. This "pre-condensation" has several advantages. Subsequent drying is quicker so that fuel is saved. The dried milk is less bulky and more soluble.

A fine spray of the condensed milk, heated to a temperature of 140°-160°F, is then forced into a chamber in which currents of hot air circulate. The dried milk falls as a powder. This must be removed from the drying chamber immediately to avoid loss of solubility. There are numerous types of spray-driers which differ from one another in the arrangements of the inlets for milk spray and hot air and in the provision for the removal of the dried milk powder.

Composition and Properties of Milk Powders.

Powders made from whole milk contain about 8 times and skimmed milk powders about 11 times the percentages of solids present in the original milk. The average composition of dried whole and separated milk and also of some other dried products obtained from milk is given in *Fig.* 16.

AVERAGE COMPOSITION OF POWDERS MADE FROM MILK AND MILK PRODUCTS.

(Figures due to Hunziker).

	Dried Whole Milk	*Dried Separated Milk*	*Dried Buttermilk*	*Dried Whey*	*Malted milk (barley malt and wheat flour added)*
	%	%	%	%	%
Water	2.5	2.5	7.0	4.0	3.0
Fat	27.5	1.0	6.5	5.0	8.0
Proteins	26.5	38.0	36.5	12.0	18.0
Lactose	37.5	50.5	35.5	70.0	12.0
Lactic Acid ...	—	—	6.0	—	—
Mineral Matter ...	6.0	8.0	8.5	9.0	3.8
Maltose + Dextrose	—	—	—	—	55.0
Fibre	—	—	—	—	0.2

The following facts are illustrated by the above figures :—

Dried separated milk is deficient in fat.

Dried buttermilk is deficient in fat and contains a considerable proportion of lactic acid due to the activity of the lactic acid organisms during the ripening of the cream.

Dried whey is deficient in fat and proteins, but is a rich source of lactose.

Malted milk contains all the milk solids but the proportions are reduced through admixture with barley malt and wheat flour. It contains the sugars, maltose and dextrose, produced from the starch of barley and wheat by enzyme action. The small proportion of fibre is from the barley and wheat.

Fig. 16.

The moisture content of milk powders must be low to avoid loss of solubility and the development of taints. The average moisture contents of spray-dried and roller-dried powders are 2.5 per cent and 5.5 per cent respectively. Milk powders readily absorb moisture from the atmosphere and must be stored in air-tight containers. Storage at high temperatures also reduces solubility.

Owing to the lower temperature to which the milk is exposed during drying, spray-dried powders have usually a higher solubility than roller-dried. Percentage solubilities are commonly 90-100 per cent for the former and 70-80 per cent for the latter. The roller-dried

product is often slightly darker in colour than the spray-dried due to some caramelisation of the lactose. The fat in the spray-dried powders exists in smaller globules than in roller-dried. Spray-dried milk after dissolving in water (*reconstituting*) can be clotted with rennet much more satisfactorily than is the case with the roller-dried product. In both processes milk with an abnormally high acidity gives a less soluble product.

The most common taint in milk powders is a gradual development of " tallowiness " due to oxidation of the olein of the milk fat. This occurs more often in the spray-dried type. The explanation of this difference seems to be the greater surface exposed to the air in the spray-dried powders owing to the much smaller size of the granules and to the fact that their porous nature permits the presence of minute air bubbles inside them. As with other milk products, exposure to light, acidity and traces of copper all accelerate the development of the taint. Recent experiments have shown that this taint can be prevented by compressing the powder into cubical blocks (usually about 30 lb. in weight), and by packing the powder out of contact with oxygen. For this purpose packing in nitrogen gas is employed. Preheating the milk to 190° F before condensation also checks the development of a " tallowy " taint. Compression into blocks was used in providing dried milks for Service use during the war of 1939-45.

OTHER DRIED MILK PRODUCTS.

Dried Buttermilk.

Buttermilk is unsuited to spray-driers as it clogs the nozzles of the sprays, but is often dried by the roller process. As it is used only for animal feeding no attempt is made to preserve the solubility. The lactic acid present (see *Fig.* 16) checks bacterial growth and so improves the keeping quality.

Dried Whey.

Whey is usually dried by the roller process. It has a number of uses including stock feeding, the manufacture of lactose, and the preparation of infant foods. It is also used in the baking and confectionery industries.

Malted Milk Powder.

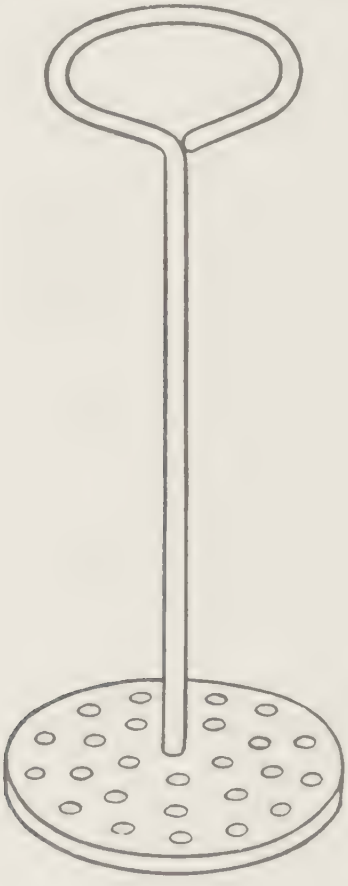
In the manufacture of this product whole milk is mixed with a mash of barley malt and wheat flour. Enzymes from the malt convert the cereal starch into sugars (maltose and dextrose). The mixture is then dried. One pound of malted milk powder contains the solids from about 2.2 pounds of milk.

The product keeps well if not exposed to moist air. Tallowiness due to fat oxidation does not occur as the fat is protected from air by the wheat gluten. Malted milk is commonly sold in tablets which are sometimes flavoured with chocolate.

SECTION IV.

THE TESTING OF MILK AND ITS PRODUCTS

Chemical tests on milk and its products are carried out for various purposes. The chemical composition of milk is of importance both on account of the existence of minimum legal standards and in connexion with the manufacture of dairy products. Milk may also be tested by chemical means to determine whether it will withstand, without clotting, heat treatment during pasteurisation or during the manufacture of condensed or dried milk. Whether pasteurisation has been efficiently carried out can be determined by chemical methods



Metal Plunger for mixing milk in the churn immediately before taking a sample.

Fig 17.

applied to the pasteurised milk. The addition of preservatives can be detected by chemical tests. A full account of all the various types of chemical examination which can usefully be undertaken on milk and milk products would fill several large volumes and would also require for its appreciation a very considerable knowledge of pure chemistry.

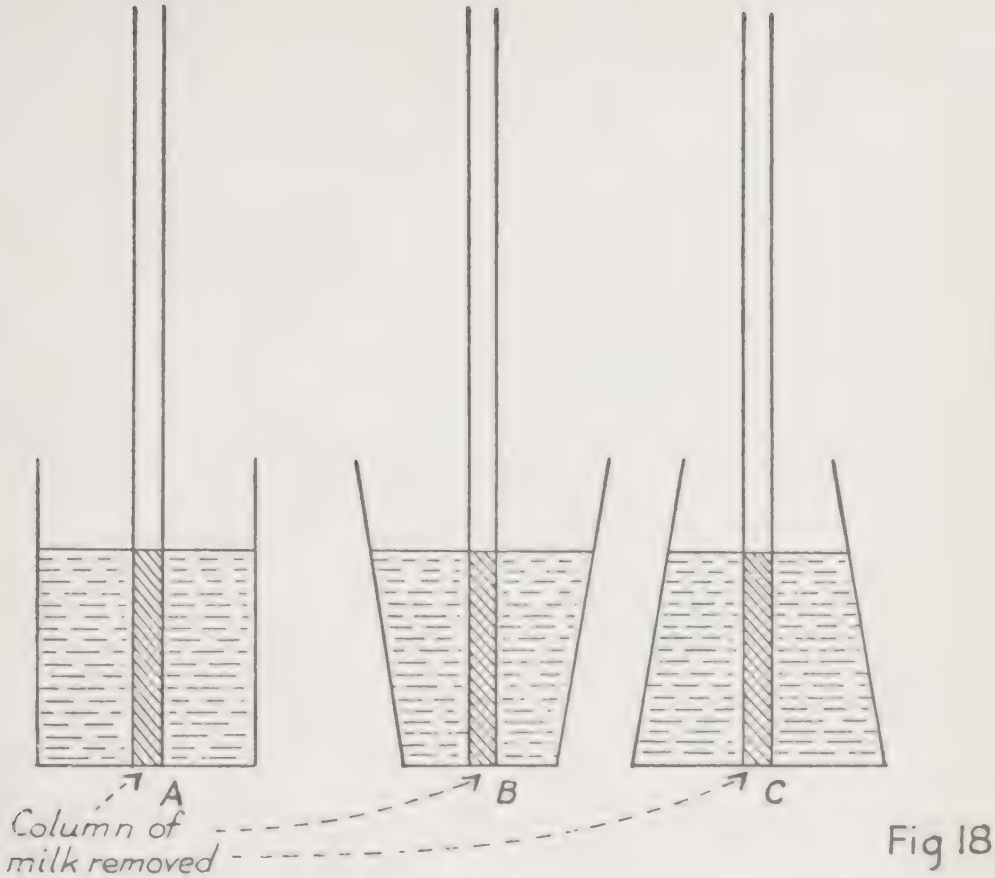


Fig 18

The Tube Method for sampling milk for chemical analysis. Success in obtaining a representative sample depends on removing the same proportion of each layer of milk, so that the vessel used must have vertical sides and a flat bottom.

In the following pages attention is confined to relatively simple tests of wide practical application and suited to the elementary student.

THE SAMPLING OF MILK AT THE FARM FOR CHEMICAL EXAMINATION

Appropriate methods of sampling are essential if reliable results are to be obtained. One of the main difficulties is the rapidity with which the fat rises to the surface. Again where a sample representative of the milk of a herd is to be obtained the difference in the volume of milk in each churn must be taken into consideration. Either of the two following methods carefully carried out will provide a representative sample.

The Plunger Method.

In this method the milk in each churn is sampled and the samples so obtained are mixed. If the quantity of milk in each churn is not the same, the amount of milk taken from a churn must be proportional

to the volume of milk in it. Thus with churns containing respectively 12, 10 and 8 gallons suitable amounts to take from them would be 6, 5 and 4 ounces.

It is essential that milk should be thoroughly mixed *immediately before* sampling. This is conveniently carried out by means of the plunger illustrated in *Fig.* 17 which is moved up and down vigorously in the milk for a few minutes. The correct quantity is then removed by means of a measure graduated in fluid ounces.

The Tube Method.

In this method a sample of each cow's milk is withdrawn from the weighing bucket by means of a tube of uniform bore open at each end. This is inserted to the bottom of the bucket, held vertically in the milk, and a sample withdrawn by closing the upper end with the thumb or finger. It is essential for the success of this method that the milk bucket should have a flat bottom and vertical sides. The samples are placed in a bottle and mixed.

This method automatically provides samples proportional to the quantity of milk. Theoretically, provided that the milk bucket is of the type indicated, mixing in the bucket is unnecessary since equal amounts are taken from each layer of milk. In practice it is advisable to mix the milk in the bucket before sampling as cream adheres to the tube more tenaciously than does milk and some loss of fat occurs from this cause. If sampling is undertaken immediately after pouring the milk into the weighing bucket, further mixing will not be required.

Preservation of Samples.

If analysis cannot be undertaken on the day of sampling, preservation is necessary. If the determination of fat only is needed it will suffice to add powdered potassium dichromate until the milk has a pale yellow colour. If the percentage of non-fatty solids is also to be estimated 1 ml. of formalin should be mixed with each quart of sample. This quantity of formalin is too small to have any appreciable effect on the result.

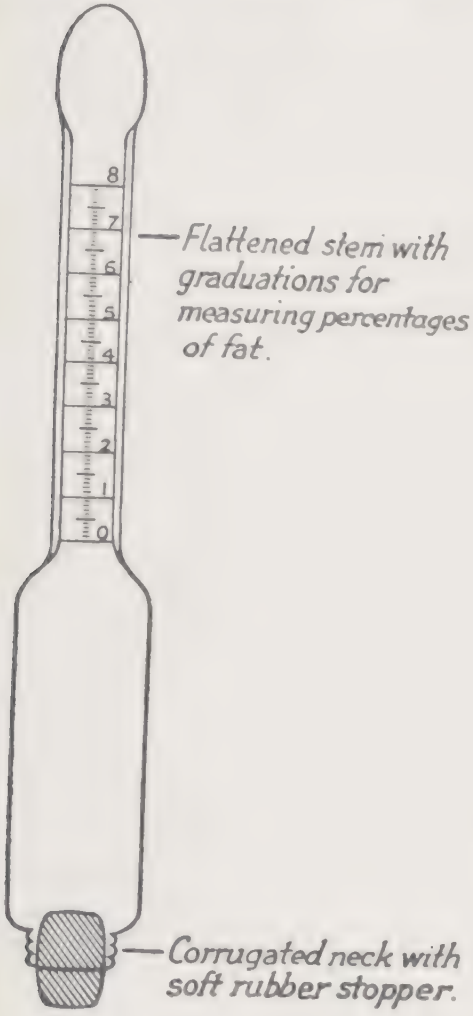
TESTS FOR ADULTERATION OF MILK

Adulteration by the addition of water can be determined in two ways. The percentage of fat and of non-fatty solids can be estimated and the results compared with the legal minimum standards for these constituents or the freezing point of the milk may be determined.

Determination of Fat.

Two methods are available for rapid fat determination; these are the Gerber and Babcock methods. The latter is the standard method in the U.S.A., but in Britain the Gerber method is employed in routine tests. In both methods the milk proteins and mineral matter are dissolved by the addition of sulphuric acid and the fat is then separated by means of a centrifuge. In the Gerber method amyl alcohol is also added prior to centrifuging to dissolve the fat. Both methods are

"empirical," that is, they give accurate results only when carried out under rigidly defined conditions. More accurate methods are available to the trained analyst, but they are lengthy and unsuited to routine work. The accuracy of the Gerber and Babcock methods when properly carried out is sufficient for all ordinary purposes.



The Gerber Tube or Butyrometer for the determination of the percentage of fat in milk.

Fig. 19.

THE GERBER METHOD.

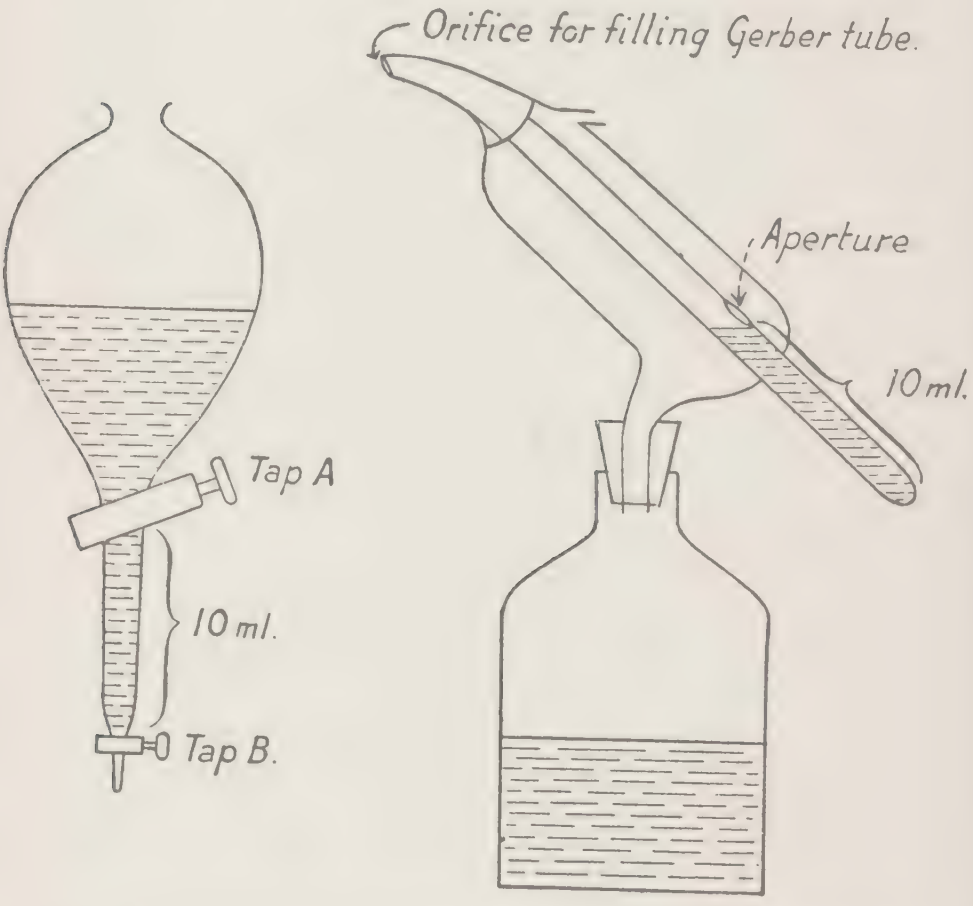
The following equipment and chemicals are needed :—

(i) *Equipment.*

(*a*) *Gerber tubes or butyrometers.* A butyrometer is illustrated in *Fig.* 19. If reliable results are to be obtained it is essential that it should conform to the appropriate British Standard Specification (B.S.S. No. 696). Modified types are available for fat determinations on skim milk, cream and cheese.

(*b*) *Automatic measure for sulphuric acid.* Alternative designs for this are shown in *Fig.* 20. It is dangerous to measure strong acids with a pipette.

(*c*) *Automatic measure for amyl alcohol.* This is similar in design to that used for sulphuric acid, but is calibrated to deliver 1 ml. Amyl alcohol can safely be measured with a pipette, but the use of an automatic measure saves time if many samples are to be examined.

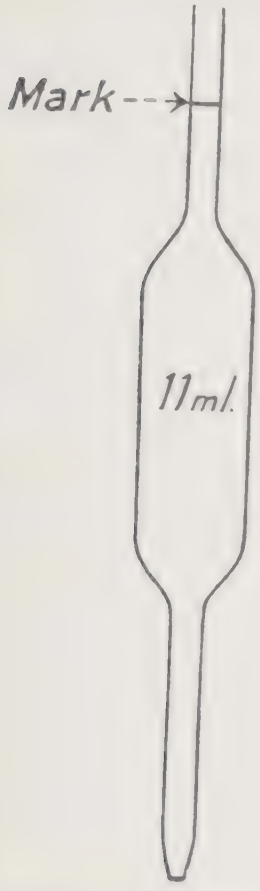


Types of automatic measures for sulphuric acid. In the type shewn on the left, Tap B is first closed and the space between the taps filled by opening Tap A. By closing Tap A and opening Tap B 10 ml. can then be run off. In the type shewn on the right the measure is first tilted to fill the part of the tube below the aperture. On returning it to the upright position excess acid drains back into the bottle. The measured 10 ml. of acid is then run into the Gerber tube through the orifice.

Fig 20

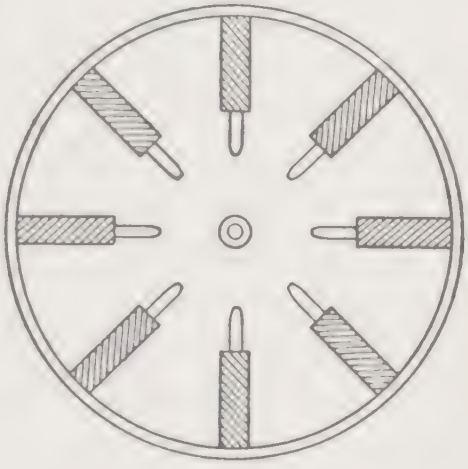
(*d*) *Pipettes for measuring out the milk sample.* A suitable type is illustrated in *Fig.* 21. These pipettes are calibrated to deliver 11 ml. of *water*. When used with milk they deliver only 10.9 ml. This volume corresponds to 11.25 grams of milk which is a suitable quantity for the Gerber test.

(*e*) *A Gerber centrifuge.* This is designed to rotate at 1,100 revolutions per minute. Types holding 8 and 16 butyrometers respectively are on the market. The butyrometers are placed with their graduated stems (in which the fat collects) towards the centre.



Pipette for measuring the milk used in the Gerber Test.

Fig 21



The Gerber Centrifuge viewed from above shewing the butyrometers in position.

Fig 22

(*f*) *Wooden stands to hold the Gerber tubes and a water bath maintained at 68°C.*

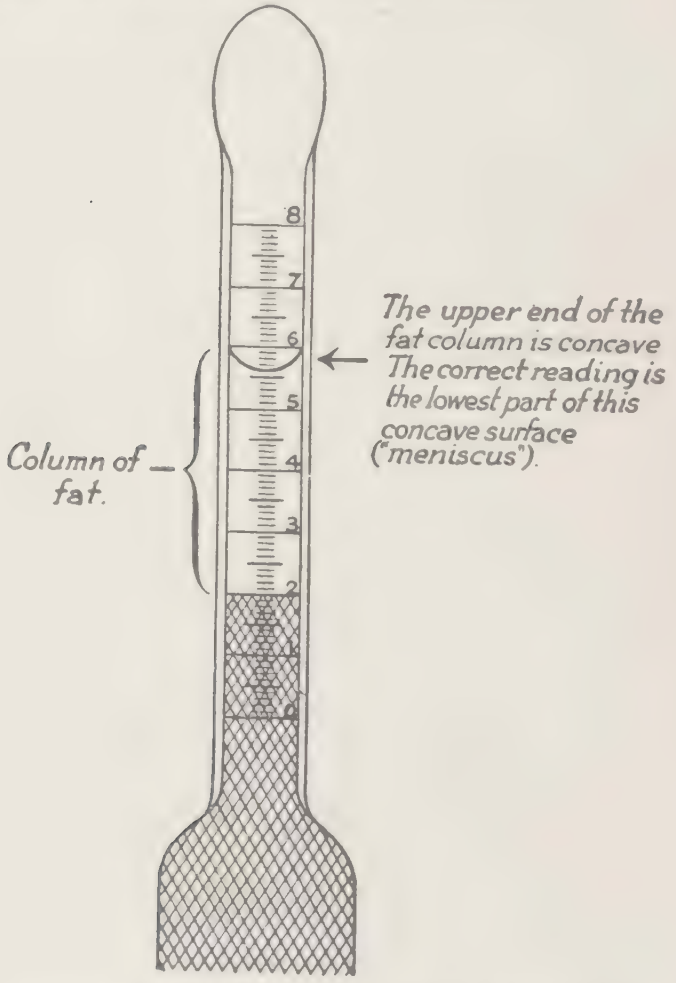
(ii) *Chemicals.*

(*a*) *Sulphuric Acid.* This must have a specific gravity of between 1.820 and 1.825 (at 60° F) and is best purchased specifically for milk testing by the Gerber method. Stronger acid will cause excessive charring of the milk proteins and incomplete separation of fat. Weaker acid is unable to dissolve the proteins completely.

(*b*) *Amyl Alcohol.* This should be purchased as suitable for milk testing by the Gerber method and should conform to the standards laid down by the British Standards Institution. Unsuitable amyl alcohol may lead to high results.

METHOD OF CARRYING OUT THE GERBER TEST ON MILK.

Place 10 ml. of the sulphuric acid in the butyrometer. Shake the milk sample thoroughly and then pipette 11 ml. of the milk on to the surface of the acid, allowing it to run slowly down the side of the Gerber tube so that it forms a distinct layer. Finally measure in 1 ml. of amyl alcohol. Care should be taken to avoid wetting the inside of the neck of the butyrometer. Then insert the rubber stopper and shake the tube until the curd is completely dissolved and no specks of curd remain. Heat is developed during shaking and this should be done cautiously. Invert the butyrometer and adjust the stopper to bring the surface of the liquid into the graduated stem.



READING THE PERCENTAGE OF FAT IN THE BUTYROMETER AFTER CENTRIFUGING.

By adjustment of the rubber stopper the lower end of the column of fat is made to co-incide with one of the graduations. At the upper end of the fat column the graduation corresponding to the lowest part of the meniscus is noted.

Thus in the case shewn in the diagram the percentage of fat is:—5.6 less 2.0=3.6.

Fig 23

Place in the centrifuge and rotate at full speed for 4 minutes. After centrifuging place the tube in the water bath at 68° C for 2 or 3 minutes and then read off the fat percentage as explained in *Fig.* 23. Homogenised milk will require a second centrifuging for 4 minutes to separate completely its very tiny fat globules.

Empty the butyrometer while still hot to avoid the solidification of fat in the graduated stem and wash immediately in hot water. The contents must not be poured into a sink unless ample water is run in at the same time; non-observance of this precaution leads to corrosion of metal pipes.

Provided that satisfactory equipment and chemicals are used and that the above instructions are rigidly observed the results obtained should not differ from those given by more accurate methods by more than 0.05 per cent.

Measuring the amyl alcoho into the butyrometer which already contains the sulphuric acid and the milk.

The Gerber Centrifuge in Use.

The butyrometers are placed in opposite positions to balance the machine and so avoid strain on the bearings.

The layer of fat in the graduated stem of the butyrometer after centrifuging.

Application of the Gerber Method to Sour Milk.

Measure 100 ml. of the curdled milk into a dry flask. Add 5 ml. of dilute ammonia solution (prepared by mixing 1 part of strong ammonia solution—specific gravity 0.88—with 3 parts of water). Shake vigorously to dissolve the curd. Determine the fat percentage of the mixture by the Gerber method and multiply the result by 1.05.

Calculation of Fat Removed from Milk.

Provided that the fat content of the original milk is known a determination of the percentage of fat in the skimmed milk enables the quantity of fat removed to be calculated accurately. The following formula is employed for this purpose :—

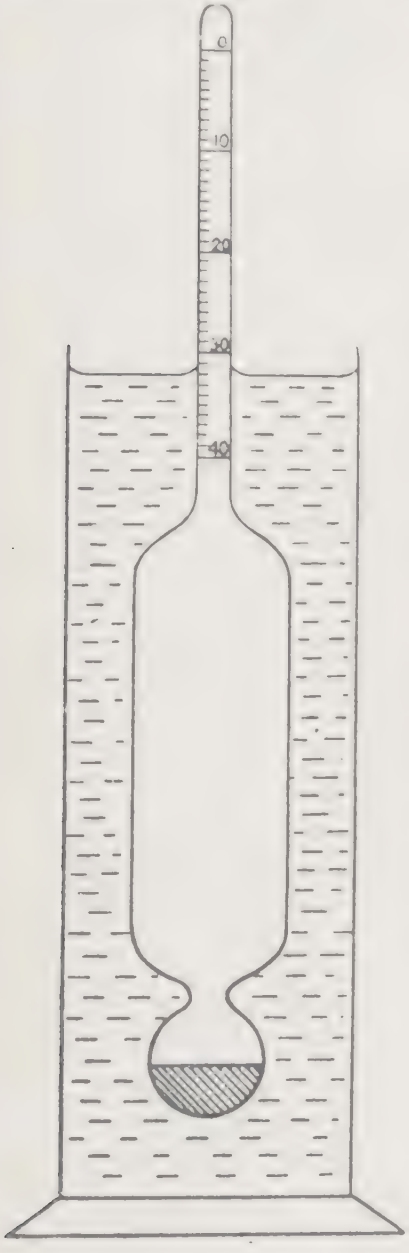
$$\text{Percentage of fat removed} \quad \frac{100\ (F_1 - F_2)}{F_1}$$

where F_1 = Percentage of fat in the original milk
F_2 = " " " " " skimmed "

Usually the fat percentage in the original milk is not known. In this case the *minimum* percentage of fat removed is sometimes calculated by substituting 3 for F_1 in the above formula. When, as is normally the case, the original milk had more than 3 per cent of fat, this substitution gives too low a result. If the original milk had less than 3 per cent of fat, the result is misleading and may suggest skimming when this did not in fact take place.

DETERMINATION OF NON-FATTY SOLIDS.

This determination is most accurately made by first estimating the percentage of total solids by evaporating to dryness a weighed quantity of milk and then subtracting the percentage of fat. This method is, however, too lengthy for routine estimations. Droop Richmond has found that a figure for non-fatty solids sufficiently accurate for most purposes can be calculated from the fat percentage and the specific gravity.



THE USE OF THE LACTOMETER IN DETERMINING THE SPECIFIC GRAVITY OF MILK.

The milk is mixed thoroughly and a glass cylinder at least 1 inch wider than the widest part of the lactometer is filled with it. The lactometer is carefully lowered into the milk so that it floats without touching the bottom or sides of the cylinder. The graduation on the lactometer scale level with the surface of the milk is noted.

Note:—(1) The lactometer scale is read *downwards*. (2) The milk surface "creeps" up the lactometer stem. The correct reading is the level of the main surface of the milk and not where the milk actually meets the stem. If the latter point is read it is usually necessary to add 0.5 to the reading to allow for this upward creep.

Fig. 24.

Determination of Specific Gravity.

For very accurate results a specific gravity bottle or a Westphal balance should be employed, but for routine tests this estimation can be very rapidly carried out with sufficient accuracy by means of a *lactometer* whose use is illustrated in *Fig.* 24. The stem of this instrument is graduated from 0 to 40 or from 25 to 35; these graduations correspond to specific gravities of 1.000 to 1.040 and 1.025 to 1.035 respectively. Thus a reading of 32 corresponds to a specific gravity of 1.032. The temperature of the milk must be read at the same time. Owing to the slight rise in specific gravity during the first few hours after milking, samples should be stored for at least 4 hours before taking lactometer readings.

There are two types of lactometer designed to give accurate readings at 60 F and 20 C respectively. If the milk is not at the standard temperature the reading must be corrected. This correction can be made by means of Richmond's scale as described later. In the case of lactometers designed for use at 60°F it can also be made by adding 0.1 to the lactometer reading for each degree above 60°F and subtracting 0.1 for each degree below 60°F.

Near view of the actometer floating in a cylinder full of milk. The "creeping" of the milk surface up the sides of the lactometer stem is clearly visible Allowance for this creeping must be made when reading the lactometer

Reading the lactometer after lowering it into a cylinder full of milk and allowing it to float without touching the bottom or sides of the cylinder.

ESTIMATION OF THE SPECIFIC GRAVITY OF SOUR MILK.

When samples cannot be examined in the fresh condition a preservative should be added as described earlier. If, however, the sample is curdled when received, the following method will give fairly accurate results :—

Add dilute ammonia solution to the sample in the proportion described in connexion with fat estimations. Shake to dissolve the curd and note the lactometer reading. This will be too low owing to the diluting effect of the ammonia. The correction to be added can be obtained by noting the lactometer reading of a sample of fresh milk, then adding 5 ml. of dilute ammonia solution to 100 ml. of this milk, mixing thoroughly and reading the lactometer in the mixture. The difference between the last two readings is the necessary correction.

The following example illustrates this method :—

Lactometer reading (corrected for temperature) in mixture of sour milk and ammonia 31.5.

Lactometer reading in fresh milk 32.0.

Lactometer reading in mixture of fresh milk and ammonia 30.5.

Correction to be added to reading in sour milk and ammonia — 32.0 less 30.5 = 1.5.

Corrected reading for sour milk 31.5 + 1.5 = 33.0.

CALCULATION OF PERCENTAGE OF NON-FATTY SOLIDS.

The following formulae give a sufficiently accurate estimation of the above.

Using a lactometer designed for 60°*F*

$$\text{Percentage Non-fatty Solids} = \frac{\text{Lactometer reading (after correction to 60 F)}}{4} + \frac{\text{percentage fat}}{5} + 0.14$$

Using a lactometer designed for 20°C

percentage Non-fatty Solids = Lactometer reading x 0.25 (corrected to 20°C) + percentage fat x 0.21 + 0.66

Use of Richmond's Scale.

This is used to correct the lactometer reading to the standard temperature and also to eliminate calculations. The scale automatically calculates the *Total Solids* from which the percentage of non-fatty solids (solids-not-fat) can be obtained by subtracting the fat percentage. In *Fig.* 25 is illustrated a scale of the type for use with a lactometer designed for 60°F.

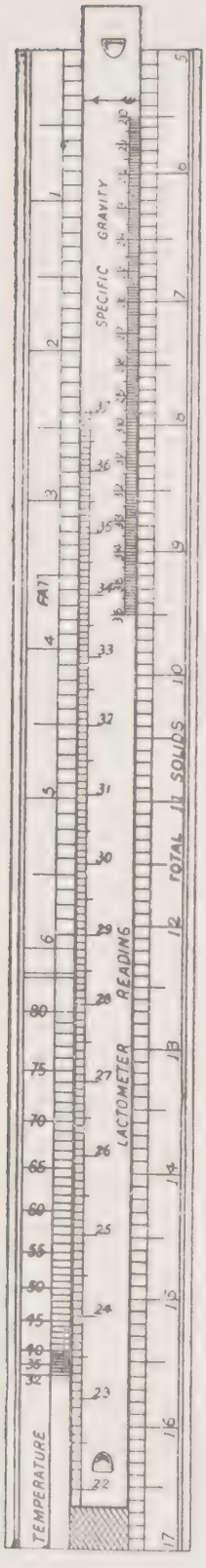


Fig. 25.

THE USE OF RICHMOND'S SCALE.

Correction of Lactometer Reading to 60°F.

Adjust the sliding, inner portion, of the scale until the observed lactometer reading is exactly opposite the graduation marked "60" on the temperature scale. The corrected reading will then be opposite the graduation on the temperature scale corresponding to the observed temperature of the milk.

Calculation of Percentage of Total Solids.

Set the arrow on the sliding, inner portion, of the scale exactly opposite the percentage of fat (as determined by the Gerber Method). The percentage of total solids will then be opposite the graduation (on the portion of the scale marked "Specific Gravity") corresponding to the corrected lactometer reading.

Calculation of Water Added to Milk.

If the percentage of non-fatty solids in the original milk is known, the percentage of water added can be calculated by means of the following formula :—

$$\text{Percentage of added water} = \frac{100\ (S^1N^1F^1 - \text{S.N.F.})}{S^1N^1F^1}$$

where $S^1N^1F^1$ = Percentage of non-fatty solids in the original milk.

S.N.F. = Percentage of non-fatty solids after the addition of water.

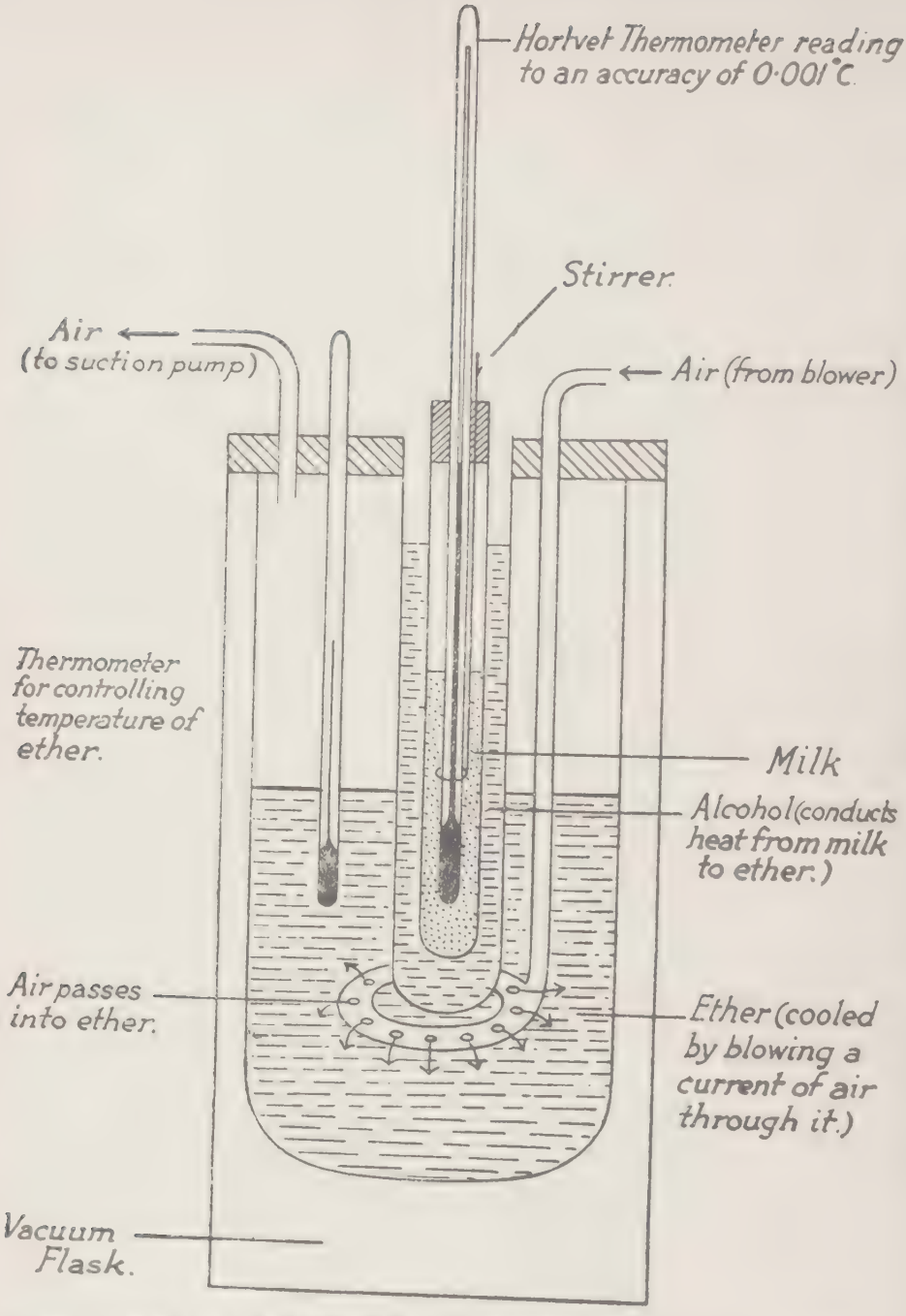
Where the percentage of non-fatty solids in the original milk is not known it is customary to calculate the *minimum* percentage of added water by substituting 8.5 for $S^1N^1F^1$ in the above formula. If the content of non-fatty solids in the original milk was above 8.5 this assumption gives too low results, while if the original milk had less than 8.5 per cent of total solids, the result of applying this assumption is unfair to the producer.

THE FREEZING POINT TEST

Detection of adulteration of milk by determining the percentages of fat and of non-fatty solids is based on the assumption that the original unadulterated milk did not fall below the minimum standards of 3 per cent fat and 8.5 per cent solids-not-fat. This assumption is not valid in every case and may occasionally result in unjust conviction for adulteration. Intensive search has therefore been made for a criterion which distinguishes genuine from watered samples and many physical and chemical properties of milk have been examined with a view to their use for this purpose. The most satisfactory property of milk from this viewpoint is its freezing point.

Milk has the same freezing point as the cow's blood and thus is unaffected in abnormal samples provided that they are genuine. Since the freezing point depends only on the constituents present in true solution—the lactose and the water-soluble portion of the mineral matter—this test does not detect removal of fat. Samples for examination must be fresh as developed acidity brings more of the mineral matter into solution and lowers the freezing point. The addition of preservatives to samples is inadmissable so that the latter must be preserved in ice prior to examination. In the milk of cows suffering from mastitis the deficiency of lactose is counterbalanced by an increase in soluble mineral matter (mainly common salt) and the freezing point is unaffected.

Fresh milk normally freezes at —0.55°C and unadulterated samples always show freezing points between the limits of —0.53° and —0.57°C. To avoid the use of the minus sign it is customary to describe the difference between the freezing point of milk and that of water as the *freezing point depression* of the milk. Thus a freezing point of —0.55°C corresponds to a freezing point depression of 0.55°C. The



DETERMINATION OF THE FREEZING POINT OF MILK IN THE HORTVET CRYOSCOPE.

The milk is placed in the central glass tube which also contains a stirrer and the bulb of a Hortvet thermometer. This glass tube fits into a metal tube containing alcohol and the metal tube is immersed in ether contained in a vacuum flask.

The ether is cooled to between —2.5 C and —3 C by blowing a current of air through it and is maintained at this temperature by regulating the air current. The milk is stirred continually and its temperature read every 30 seconds. Its temperature falls at first below its freezing point, but rises rapidly to the true freezing point when freezing commences. It then remains steady for a few minutes before beginning to fall again. This steady temperature is noted.

Fig 26

percentage of water added can be calculated from the following equation :—

$$\text{Percentage Added Water} = \frac{100\ (t - t^1)}{t}$$

where t = F.P. depression of unwatered milk.
t^1 = F.P. depression of watered milk.

For example if the F.P. depression of the original milk was 0.55°C and that of the watered milk 0.45°C, the percentage of water added is :—

$$\frac{100\ (0.55 - 0.45)}{0.55} = 18.2$$

Where the F.P. depression of the original milk is unknown it can—without the possibility of injustice to the producer—be taken as 0.53°C. The use of this assumption will generally underestimate slightly the percentage of water added.

The determination of the freezing point of milk is carried out in a *Hortvet Cryoscope* whose use is illustrated in *Fig.* 26.

It will be noted that the temperature of the milk falls below its freezing point before freezing occurs. This phenomenon is known as *super-cooling*. Excessive super-cooling takes place if the ether bath surrounding the milk falls below —3°C and this leads to inaccurate results. Occasionally there is a considerable delay before the milk commences to freeze. In such cases a minute crystal of ice is dropped into it while its temperature is maintained at about 1°C below its probable freezing point. This usually results in immediate freezing with a consequent rise of temperature to the freezing point.

It is generally necessary to correct the reading to allow for a small error in the Hortvet thermometer. The latter may be submitted to the National Physical Laboratory for calibration; this laboratory issues a certificate indicating the correction. Alternatively Hortvet thermometers may be calibrated by the use of sugar solutions whose freezing points are accurately known. Various formulae have been suggested for the correction of readings taken on milk containing developed acidity. None of these is entirely satisfactory.

The Hortvet Cryoscope being used to determine the Freezing Point of milk.

THE DETERMINATION OF DEVELOPED ACIDITY IN MILK

The development of lactic acid in milk renders it unsuitable for processing on account of the risk of clotting when heated. A number of tests for acidity are in daily use by manufacturers of milk products, and are employed to indicate milk which must be rejected through this risk. They can be conveniently classified into simple tests for incipient souring and determinations which give a *measure* of the acidity.

Simple Tests for Souring.

(1) *The Boiling Test.* Milk will usually clot on boiling when the total acidity (expressed as a percentage of lactic acid) has risen to 0.26. This figure is, however, well above the safety limit for condensing and drying plants so that this simple test has only a limited use.

(2) *The Alcohol Test.* Milk is shaken with an equal volume of a mixture of 68 parts of alcohol and 32 parts of water (or 72 parts of methylated spirits and 28 parts of water). If the total acidity (expressed as a percentage of lactic acid) has risen to 0.21 specks of casein usually appear on shaking.

(3) *The Alizarin-Alcohol Test.* For this test the alcohol-water mixture prepared as above is saturated with the dye *alizarin.* The colour on shaking with an equal volume of milk gives an approximate indication of the acidity. Milk from cows suffering from mastitis is usually slightly alkaline and can also be distinguished by the colour developed. The following table indicates the usual relations between the acidity, the colour developed and the type of clot.

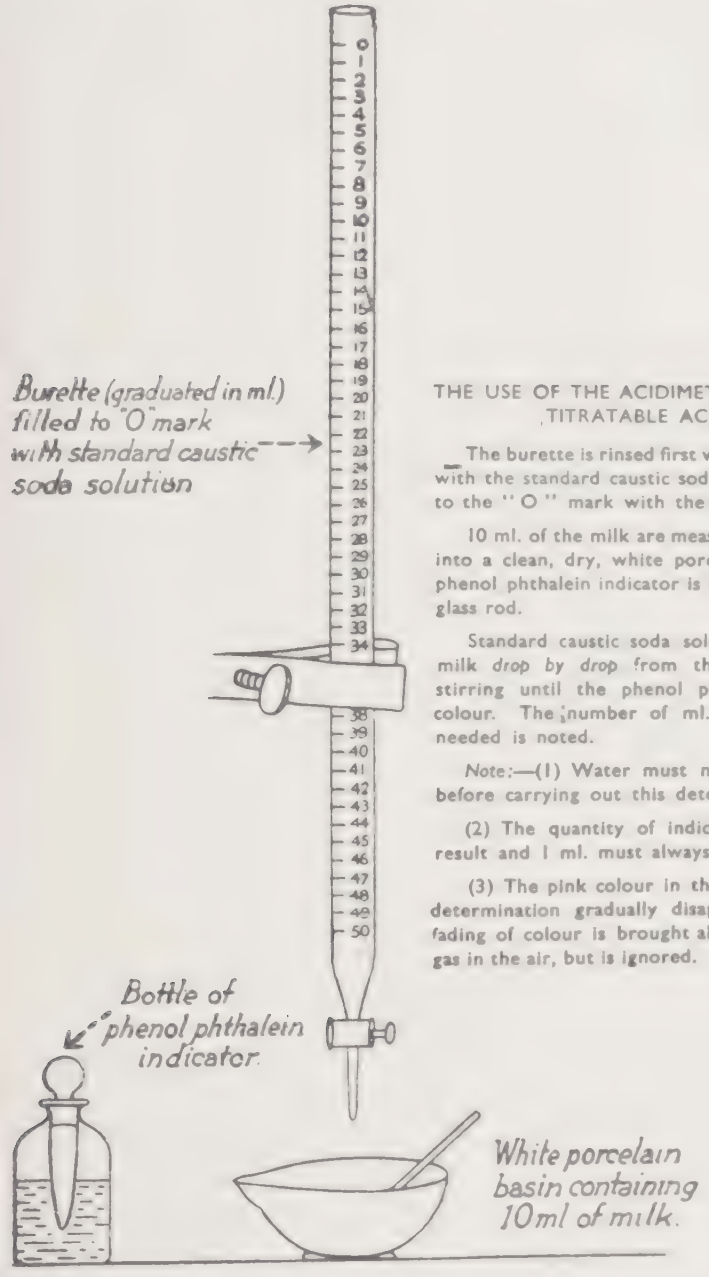
Total Acidity (expressed as lactic acid) %	*Colour developed.*	*Type of clot.*
0.16	Lilac red	No clot
0.18	Pale red	No clot
0.20	Brownish red	Very fine particles
0.22	Reddish brown	Fine particles
0.25	Brown	Small flakes
0.27	Yellowish brown	Large flakes
0.31	Brownish yellow	Large flakes and sour smell
0.36	Yellow	Coagulates
Alkaline	Violet	Fine flakes

If rennin-forming bacteria are present, cheesy flakes and a dark brick red colour appear at acidities between 0.16 and 0.18.

Although these simple tests have a limited value in indicating samples in which a considerable development of acidity has occurred, they lack exactness. Hence they are now largely superseded by determinations of *titratable acidity* by means of the *acidimeter* described below.

Determination of Titratable Acidity.

This determination estimates the *total acidity*, that is, the combined natural and developed acidity, and is carried out by means of the acidimeter whose use is illustrated in *Fig.* 27.



THE USE OF THE ACIDIMETER TO DETERMINE THE TITRATABLE ACIDITY OF MILK.

The burette is rinsed first with distilled water and then with the standard caustic soda solution. It is then filled to the "O" mark with the latter solution.

10 ml. of the milk are measured by means of a pipette into a clean, dry, white porcelain basin. 1 ml. of th phenol phthalein indicator is added and stirred in with glass rod.

Standard caustic soda solution is then run into the milk *drop by drop* from the burette with continual stirring until the phenol phthalein is faintly pink in colour. The number of ml. of caustic soda solution needed is noted.

Note:—(1) Water must not be added to the milk before carrying out this determination.

(2) The quantity of indicator added influences the result and 1 ml. must always be used.

(3) The pink colour in the milk at the end of the determination gradually disappears on standing. This fading of colour is brought about by the carbon dioxide gas in the air, but is ignored.

Fig 27.

The acidity of the milk can be expressed either as a percentage of lactic acid or as a number of " degrees of acidity." In the former case *one-ninth normal* caustic soda is used in the burette and the number of millilitres of caustic soda required is *divided* by 10.

e.g.—If 10 ml. of the milk required 1.8 ml. of one-ninth normal (N/9) caustic soda, the percentage of lactic acid is 0.18. In milk-processing plants, milk with a total acidity corresponding to more than 0.19 per cent of lactic acid is often rejected. This may very occasionally be unjust to producers whose milk has a high natural acidity.

For the determination of the number of degrees of acidity *one-tenth normal* caustic soda is used and the number of millilitres required is ***multiplied*** by 10.

e.g.—If 10 ml. of the milk required 2.0 ml. of one-tenth normal (N/10) caustic soda the number of degrees of acidity is 20.

The Acidimeter in use for determining the titratable acidity of milk. One-ninth normal caustic soda solution is being run into the milk drop by drop.

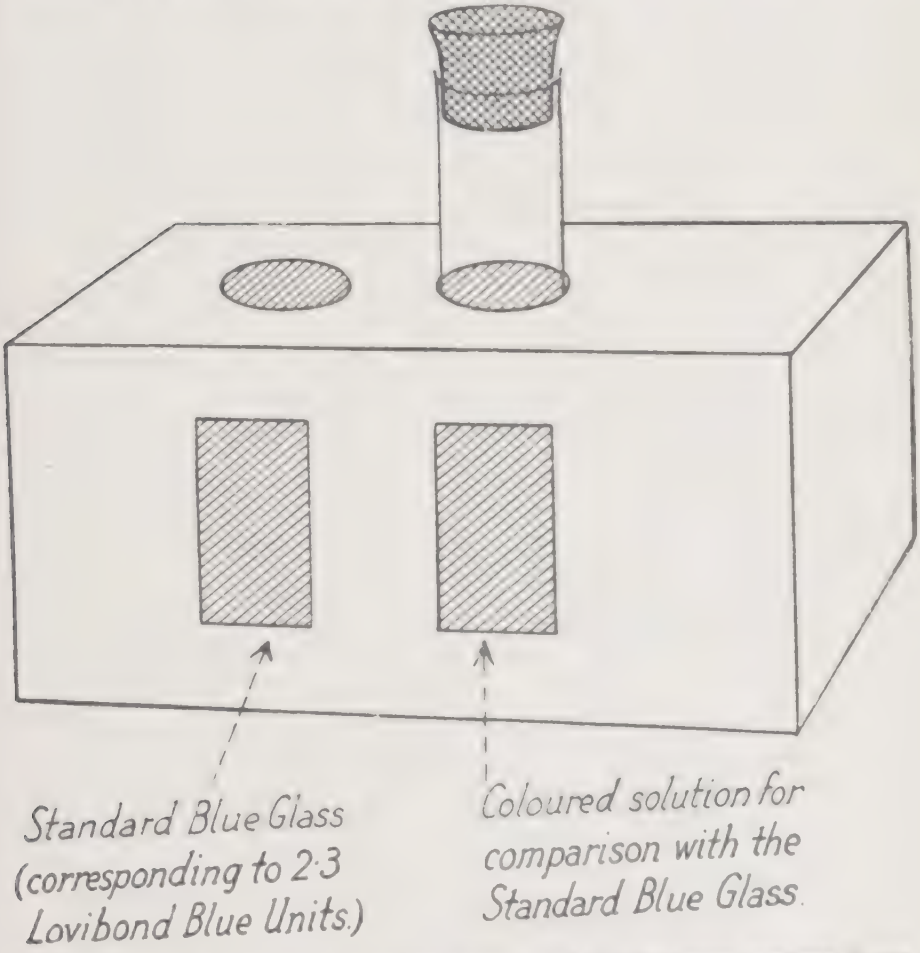
THE PHOSPHATASE TEST FOR EFFICIENCY OF PASTEURISATION

This chemical test has proved of great value in the control of pasteurisation. Prior to its discovery the efficiency of the process could be checked only by the use of thermographs (recording thermometers) in the milk during holder pasteurisation and this did not overcome the difficulty that some parts of the milk tank might not reach the necessary temperature for the requisite time. In fact " pockets " of milk insufficiently heated were of common occurrence. This test has made it possible to check the pasteurised milk itself and by its use inefficient plants have been detected in large numbers and the necessary steps taken to put matters right. Subsequent admixture with raw milk can also be detected even if this amounts only to 0.2 per cent.

The test depends on the fact that the enzyme *phosphatase* is destroyed both in the holder and in the high-temperature-short-time pasteurising processes provided that these are properly carried out.

Phosphatase has the power of decomposing organic compounds containing phosphate. In the test the phosphate compound used is disodium phenyl-phosphate (the so-called "buffer-substrate") and this is decomposed by phosphatase with the liberation of phenol. The latter gives a blue colour with Folin and Ciocalteu's phenol reagent. The intensity of this colour indicates the phenol liberated and hence the activity of the phosphatase, and is measured by means of standard blue glasses marked in "Lovibond units."

For reliable results the test must be carried out under rigidly defined conditions. Apparatus and reagents must conform to prescribed standards. Reagents must be stored in glass stoppered bottles in a cool, dark place. All glassware must be cleaned thoroughly with chromic acid before use. Rubber stoppers used in the test must be tested for phenol before use and freshly boiled distilled water must be employed. Contamination of pipettes with saliva must be avoided as this contains phosphatase, and tests must not be undertaken in direct sunlight. The following is a simplified form of the official test.



The Lovibond "Limitester" for the estimation of the blue colour developed in the Phosphatase Test.

Fig 28

Details of the Test.

If samples are kept at room temperature before examination they must be tested within 18 hours of pasteurisation. Alternatively they may be stored in a refrigerator, but must be heated to room temperature before examination. All tests should be carried out in duplicate and the two results compared.

Place 10 ml. of the buffer-substrate solution in each of 2 tubes and to each add 0.5 ml. of the well-mixed milk. Add 3 drops of chloroform to each tube to check bacterial growth. Insert rubber stoppers (previously sterilised in boiling water) and mix the contents of the tubes. Maintain for 24 hours at 37°C in an incubator or water-bath.

Cool to room temperature, add 4.5 ml. of Folin and Ciocalteu's reagent to each tube, allow to stand for 3 minutes and filter into clean tubes. To 10 ml. of each filtrate add 2 ml. of a 14 per cent solution of sodium carbonate and mix well, Place the tubes in boiling water for 2 minutes to develop the colour and then cool to room temperature. If not clear, filter the contents.

Compare the blue colour with the standard blue glass in the Lovibond Limitester illustrated in *Fig.* 28 or estimate the blue colour in a Lovibond Tintometer. Samples which produce a colour corresponding to more than 2.3 Lovibond blue units have been inefficiently pasteurised or have subsequently been mixed with raw milk.

THE DETECTION OF PRESERVATIVES AND OF ADDED COLOURING MATERIALS IN MILK

The addition to milk of preservatives or of colouring materials is illegal. The following simple tests will detect some substances of these two types.

Hydrogen Peroxide.

This preservative gradually disappears from milk as it is decomposed into water and oxygen by milk enzymes (especially catalase). If less than 0.1 per cent has been added it is not likely to be detected in milk more than 24 hours old. Larger amounts will remain and may be detected. The following test depends on the oxidation of a dye (paraphenylene diamine) by the milk enzyme *peroxidase* in the presence of hydrogen peroxide. This enzyme is, however, destroyed in milk containing hydrogen peroxide so that it is necessary to add fresh milk to supply it.

To about 10 ml. of the suspected milk add an equal volume of fresh raw milk and then a few drops of a 2 per cent solution of paraphenylene diamine. Mix well. The development of a blue colour indicates the presence of hydrogen peroxide.

Formalin.

The following test is useful only for *traces* of formalin. If a negative result is obtained, dilute the milk with ten times its volume of water and repeat the test.

To 10 ml. of the suspected milk add 0.5 ml. of a 1 per cent solution of ferric chloride and then 10 ml. of water. Pour sulphuric acid (of the strength used in the Gerber fat test) slowly down the side of the tube so as to form a distinct layer at the bottom. If formalin is present a violet coloured ring appears at the junction of the two liquids.

Benzoic and Salicylic Acids.

Place a little of the suspected milk in a test tube and add a few drops of a solution of ferric chloride. The mixture develops a buff colour in the presence of benzoic acid or a violet colour if salicylic acid is present.

Boric Acid.

Take about 20 ml. of the suspected milk in a beaker and add 2 ml. of phenol phthalein indicator. Then with constant stirring run in drop by drop from a burette N 9 or N 10 caustic soda solution until the indicator is faintly pink.

Then divide the mixture into two equal parts. Add to the first an equal volume of distilled water and to the second an equal volume of a 50 per cent solution of glycerol in water. If boric acid is present the colour in the tube containing the glycerol will be much fainter than that in the tube to which water has been added.

For this test it is essential that the glycerol used should be neutral (*i.e.* neither acid nor alkaline).

Carbonate and Bicarbonate of Soda.

These are not strictly preservatives as they do not check the growth of bacteria, but they neutralise the lactic acid formed and so prevent curdling. Since they permit the development of bacteria in the milk, their use is particularly undesirable. They can be detected as follows :—

Dilute 50 ml. of the suspected milk with 250 ml. of water. Warm and add alcohol. Filter off the precipitate, evaporate the filtrate to half its bulk and test with red litmus paper. If either of these substances is present the litmus paper will turn blue.

Annatto.

The object of adding this yellow colouring material is to give milk a " richer " appearance. If this is suspected proceed as follows : —

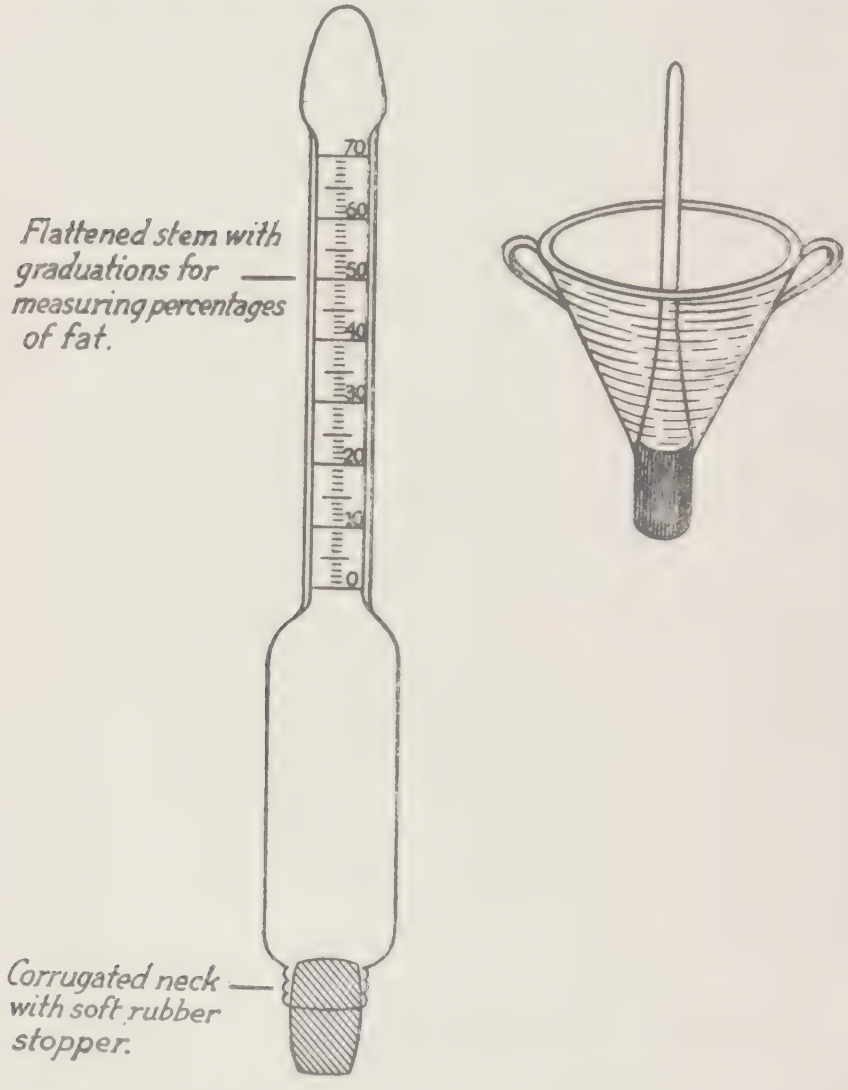
Place a small piece of red litmus paper in the milk and add just enough of a solution of carbonate of soda to turn the paper blue. Then put in a small portion of cotton wool or filter paper, and leave for a few hours. If annatto is present the cotton wool or filter paper will be stained brown and the colour will change to pink on adding a solution of stannous chloride.

When using any of the above tests it is advisable to repeat the test on a sample of genuine milk and to compare the result with that obtained with the suspected milk.

DETERMINATION OF PERCENTAGE OF FAT.

This estimation is most conveniently carried out by means of a modification of the Gerber Method used for milk. A special type of butyrometer, illustrated in *Fig.* 29, is needed. The stem of this is graduated to give the percentage of fat when 5 grams of cream are used.

Weigh 5 grams of cream into a small weighing funnel provided with a stopper and suspended from the arm of the balance. Then insert the funnel into the neck of the butyrometer, withdraw the stopper and allow the cream to run into the butyrometer. Wash any



Butyrometer and Weighing Funnel used in the Estimation of Fat in Cream

Fig 29

cream remaining on the stopper and in the funnel into the butyrometer with 6 ml. of hot distilled water. Run 10 ml. of sulphuric acid (of the strength used in the Gerber test for milk) on to the top of the cream and follow with 1 ml. of amyl alcohol (as for milk) and with sufficient warm distilled water to bring the level almost up to the shoulder of the butyrometer.

Mix thoroughly, insert in a water bath at 60° C for a few minutes and then centrifuge for 4 minutes. Then immerse in water at 68° C for 2 or 3 minutes and read off the percentage of fat. In some cases a second centrifuging may be needed to complete the separation of fat.

Determination of the Acidity of Cream.

This determination may be needed in connexion with cream ripening for buttermaking. The titratable acidity may be determined similarly to that of milk. With thick cream it may be necessary to wash the pipette with water after use and to add the washings to the cream in the porcelain basin, but as little water as possible should be employed. Very thick cream cannot be measured with a pipette. In this case weigh 10 grams of cream in the porcelain basin and add just enough water to make the cream sufficiently fluid to titrate.

BUTTER

Sampling of Butter.

Take several borings with a butter sampler from various parts of the bulk. Each boring should extend to the centre. Place the cores in a well stoppered bottle.

Determination of Percentage of Fat.

For this estimation a butyrometer of the type used for cream is employed. Weigh the butyrometer, place in it about 2.5 grams of butter and re-weigh. The weight of butter used can then be obtained by subtraction. Prepare a mixture of equal volumes of water and sulphuric acid of Gerber strength (by pouring the acid into the water and *not vice-versa*). Run sufficient of this mixture into the butyrometer to bring the contents on to the graduated scale when the stopper is inserted and the butyrometer inverted (after allowing for the 1 ml. of amyl alcohol to be added subsequently). This quantity can be determined only by trial and Gerber tubes should be marked at the appropriate level.

Add 1 ml. of amyl alcohol (as for milk), place in a water bath at 60°C for a few minutes, and then centrifuge for 4 minutes. Immerse in water at 68° C for 2 or 3 minutes and read off the percentage of fat. Multiply the percentage of fat read off by the following factor :—

$$\frac{5}{\text{Weight of butter used}}$$

Test for Rancidity in Butter.

Rancidity in butter is usually of the " oxidative " type due to the oxidation of the olein. This can be detected by the following test :—

Place about 10 ml. of melted butter fat in a test tube. Add 10 ml. of strong hydrochloric acid and 10 ml. of Kreis reagent (a 0.1 per cent solution of phloroglucinol in ether). Close the tube with a rubber stopper and shake vigorously for half a minute. Allow to stand and

note the colour of the acid layer. A deep red colour appears in this layer if oxidative rancidity has developed in the butter.

CHEESE

Determination of the Strength of Rennet.

This determination is carried out both on rennet extracts supplied as liquids and on rennet powders. In factories, milk powders after reconstituting are generally used for standardising rennet, but separated milk is also suitable for this purpose. The strength of a rennet extract or powder is expressed by the number of parts of milk which 1 part of the rennet preparation can clot in 40 minutes at 35° C. Since the rate of clotting of milk depends on the temperature and the acidity of the milk as well as on the strength of the rennet both temperature and acidity must be fixed. The determination is carried out as follows :

Dilute the rennet preparation by mixing 5 ml. of rennet extract or 0.5 gram of rennet powder with sufficient distilled water to give a final volume of 100 ml. Heat 100 ml. of separated milk to 35°C and maintain at this temperature. For reliable results this separated milk should have an acidity corresponding to 0.18 per cent of lactic acid. Add 1 ml. of the diluted rennet stirring during the addition and continue gentle stirring until clotting takes place. Note the exact time needed for clotting.

Calculate the rennet strength from the following formulae :—

For liquid rennet extracts

$$\text{Strength} = \frac{80{,}000}{\text{Number of minutes required for clotting}}$$

For rennet powders

$$\text{Strength} = \frac{800{,}000}{\text{Number of minutes required for clotting}}$$

Sampling of Cheese.

Insert the cheese sampler from a point halfway up the side and take a boring to the centre of the cheese. From the core so obtained remove the outside three-quarters of an inch (including the rind) and use this to plug the hole. Seal the cut by rubbing gently with a little cheese.

Determination of Percentage of Fat.

Weigh 5 grams of cheese into a butyrometer of the type used for cream. A small funnel fitting into the neck of the butyrometer can be used to assist in inserting the cheese. Prepare a mixture of equal volumes of sulphuric acid (of Gerber strength) and water and while still hot, add sufficient of this mixture to bring the liquid into the graduated stem of the butyrometer when this is stoppered and inverted (after allowing for the 1 ml. of amyl alcohol to be added subsequently).

Add 1 ml. of amyl alcohol (as for milk), mix thoroughly and place in a water bath at 70° C. Shake at intervals until the cheese has dissolved. Centrifuge for 4 minutes and read off the percentage of fat.

BACTERIOLOGY OF MILK AND MILK PRODUCTS

By

J. M. Goldie, B.Sc., N.D.A., N.D.D., C.D.D. (Hons.)

SECTION I.—RAW MILK.

MILK AND ITS SECRETION.

Milk is a fluid secreted by the mammary glands of the female mammal for the nourishment of her young. Under normal conditions the release of milk does not occur until after the young is born, that is, after parturition. The secretion of milk in a cow's udder is a highly complicated physiological process and is governed by the activity of chemical substances known as "hormones." The development of the udder and the initial secretion of milk are controlled by hormones liberated by the ovaries of the cow when she becomes pregnant. These hormones (*oestrogens*) may be prepared synthetically and, when injected into barren cows and virgin heifers can often induce them to come into lactation.

All the constituents of the milk are conveyed to the udder via the blood vessels, whose many branches diffuse throughout the whole udder and intimately contact all the milk-secreting cells. The blood system also supplies the "milk" cells with the large volumes of oxygen which they require and for this reason, there is a large and rapid flow of blood through the udder. About 400 gallons of blood pass through the udder for the production of every gallon of milk. The actual formation of milk takes place in the "milk" cells of the "alveoli" of the udder. These milk-secreting alveoli are microscopic in size and consist of minute spaces lined by the cells which make the milk. They constitute the principal substance of the udder, but in between them there are strands of "connective tissue" which support and protect the delicate alveoli. The various constituents of the milk pass through the cells and collect as tiny milk droplets in the spaces or "lumens" of these alveoli. The alveoli join up like fingers on a hand and form small ducts, which in turn coalesce to form larger ducts which eventually lead to the *milk sinus* or *milk reservoir*. This is composed of soft, spongy tissue and is situated round the base of the teat. The milk reservoir in turn leads on to the *teat canal*, which is provided with "sphincter" muscles, that is, regions of circular muscle fibres which only relax and let through milk when under pressure, as when the cow is being milked or suckled by a calf. Normally, they are fully contracted and keep the teat closed so preventing loss of milk.

Each quarter has its own reservoir and teat system. Although the four quarters are contained in the one structure, they are in no way connected. A fibrous wall runs longitudinally dividing the udder into two left-hand and two right-hand quarters, but there is no division of this nature between the fore and hind quarters. Proof that each quarter is a separate unit is to be seen in cows suffering from mastitis in one or more quarters, where the other quarters are milking normally. The hind quarters on the average, secrete 60% of the milk and the fore quarters only 40%.

DIAGRAMMATIC SECTION OF COW'S UDDER SHOWING FORE AND HIND QUARTERS

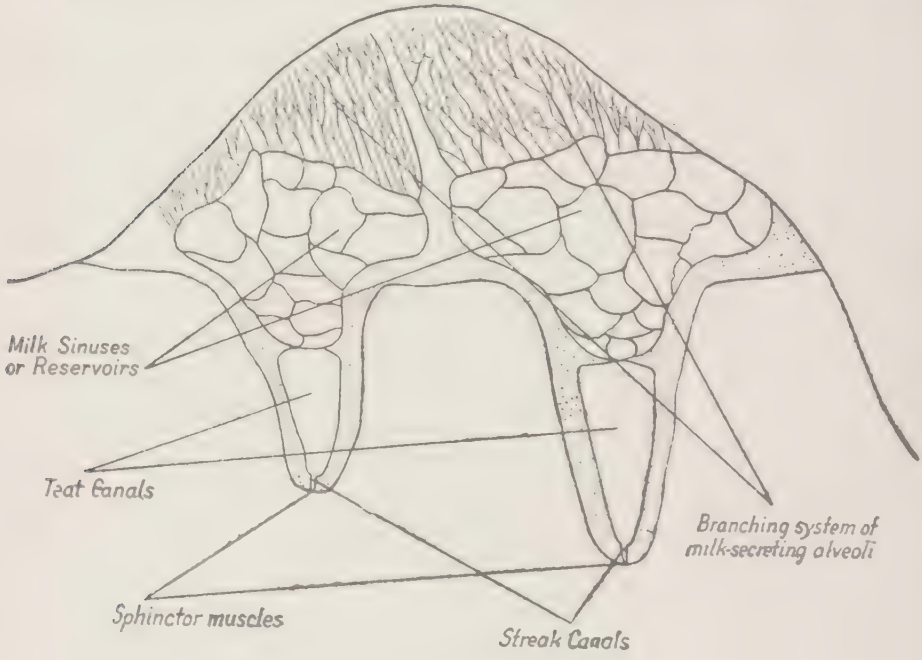


FIG. 30.

Between milkings, the milk slowly accumulates in the alveoli of the udder, although small amounts of it pass on and collect in the milk reservoir. If the next milking is delayed too long and these alveoli become too full, milk may be absorbed into the cow's blood system, which means a loss of potential milking capacity. This is one of the reasons for regular milking at equal intervals.

The letting-down of milk is simply the " squeezing " of the milk out of these alveoli and into the milk reservoir. This is brought about by contraction of the alveolar cells. It is purely a reflex action and is largely involuntary. It is controlled by the action of a hormone known as *oxytocin*, which is secreted by the posterior pituitary body at the base of the cow's brain. Under natural conditions, the butting and nuzzling of the calf prior to sucking provides the sensory stimulation necessary to promote secretion of this hormone. The oxytocin is carried to the udder in the blood-stream and in about one minute after the initial stimulation, the cow will let her milk down.

In the cowshed, this initial stimulation may be provided by a number of factors, such as handling of the teats and udder when they are being washed prior to milking, and the clatter of utensils and of milking-machine units. Any upset from normal, however, or any fright the cow receives is sufficient to prevent secretion of oxytocin and therefore to prevent let-down of milk. The best stimulus is found to be the massaging of the teats and udder with a cloth wrung out in water at 125° F. This approaches very closely to the action and heat of the calf's mouth. Milking should begin within about a minute

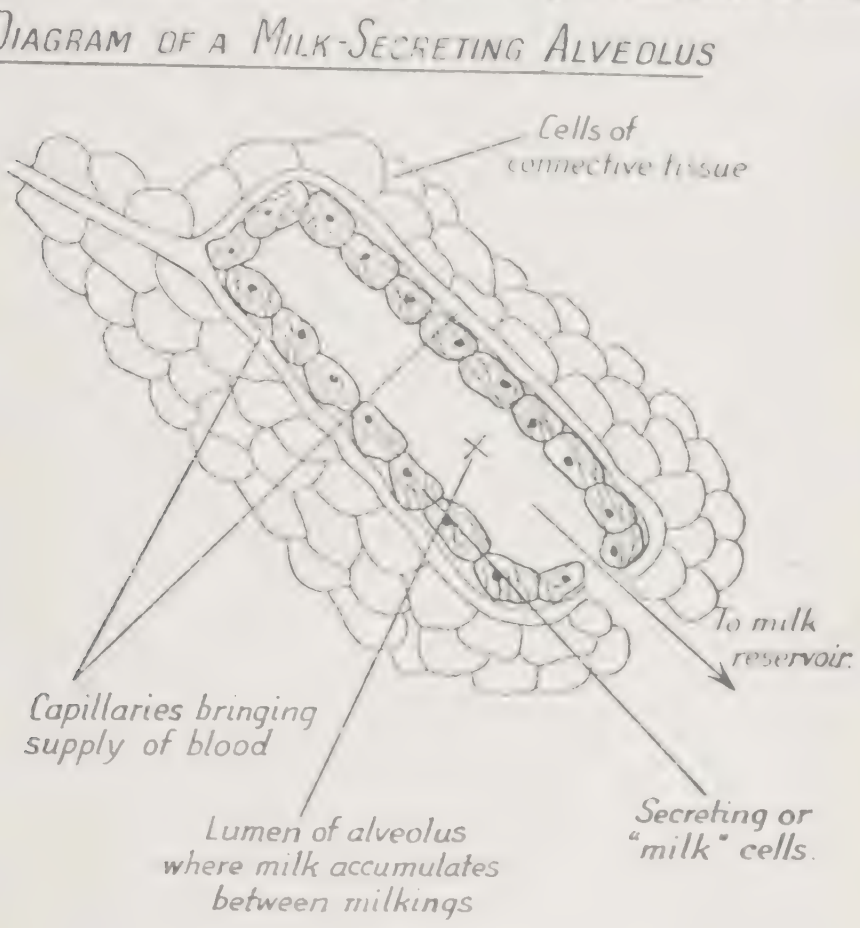


FIG. 31.

of massaging the udder. At this stage the milk is fully let down and even high yielders can be completely milked out in a few minutes. The reason for this recommendation is that the action of the oxytocin is then at its height and the milk is being actively exuded from the alveoli of the udder. If milking is delayed too long, the effect of the hormone soon wears off, however, and the cow becomes more difficult to milk. No more oxytocin is secreted until a fresh stimulus is supplied and then the process starts all over again.

The modern theory of quick milking, as first advanced by Professor Petersen of Minnesota, is based on this release of the "let-

down" hormone subsequent to stimulation by the warm massage. The principle is that all the milk should be extracted from the cow while this hormone is still active in promoting "let-down". This theory is abundantly confirmed by practical results. Cows can be milked rapidly and practically to completion. There is very little residual milk left in the udder as the effect of the hormone is only beginning to wear off when milking is finished.

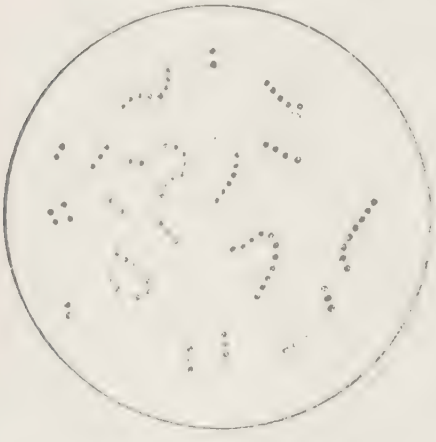
BACTERIA AND FUNGI

These micro-organisms are frequently called plants, but may also be considered as a third class of living things, intermediate between plants and animals. Unlike the green plants, they contain no chlorophyll and cannot make complex substances from simple compounds like carbon dioxide and water. They are thus either *parasites* or *saprophytes* according to whether they live on living or dead materials.

Structure of Bacteria.

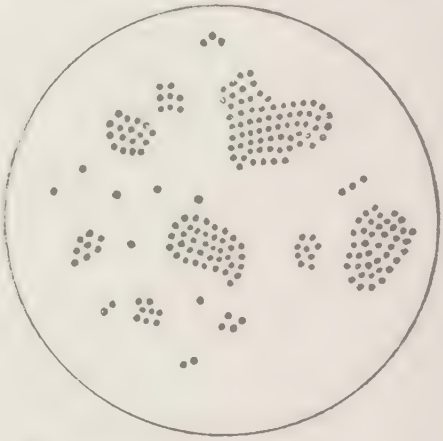
Bacteria are microscopic, unicellular organisms, with cells of various shapes, such as spherical, cylindrical and spiral. In size, they vary from about 1/2,000 m.m to 5/1,000 m.m. They reproduce by simple division, or in some types, by asexual spores, but they have no special spore-bearing structures. Bacteria have no nuclei, as have fungi and all other organisms. Most of them can move by means of hair-like "flagella".

Bacteria are very widely distributed and are always present in soil, air and water, and on the skin, but never in the blood and tissues of healthy animals. They are of great economic importance as some can cause disease, such as tuberculosis, mastitis and anthrax. Others cause fermentation. Natural rotting is brought about by the putre-



Streptococci : spherical cells in chains of varying lengths Magnified about 1000 diameters

FIG. 32.



Micrococci : spherical cells in irregular clumps Magnified about 1000 diameters

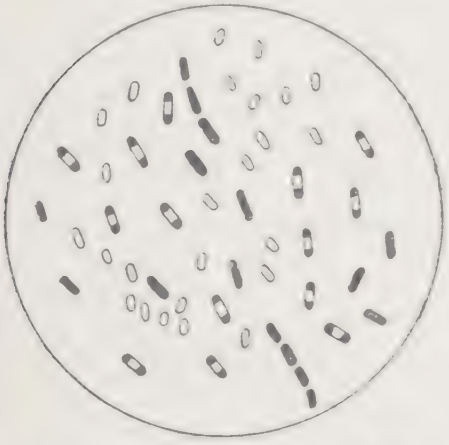
FIG. 33.

factive bacteria. The great majority of bacteria are quite harmless to Man and his plants and animals, and some are directly essential, as for instance, the nitrifying bacteria in the soil.

Bacteria are named according to the shape of their cells and their type of growth :—

(*a*) The *cocci* have spherical cells. If they grow in long chains, they are called *streptococci*. If they form clumps like bunches of grapes, they are called *staphylococci* and *micrococci*, and if they grow in groups of four or eight, they are called *tetracocci*.

(*b*) The *bacilli* are cylindrical and often form spores inside their cells. Anaerobic bacilli, that is, those which do not require free oxygen for their growth, and whose spores are so large that they distort the cells, are known as *clostridia*. Cylindrical organisms which do not form spores are known as *bacteria*.



Bacilli : rod-shaped cells often containing spores. Spores not larger than the mother cells. Magnified about 1000 diameters.

FIG. 34.

Clostridia rod-shaped cells frequently with spores. Spores larger than mother cells. Magnification × 1000

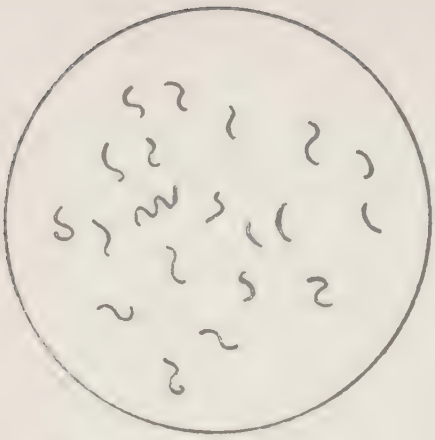
FIG. 35.

(*c*) The *spirilla* are bent, wavy, spiral or curved forms.

In certain bacteria, *capsule formation* occurs, that is, the cells become covered with a sticky gelatinous film. Owing to this film, these types can "seed" on to dairy utensils and are very difficult to remove. This is particularly serious with those bacteria which can cause taints or physical defects in milk. Bacteria which form spores do so under adverse conditions and these spores are very resistant to heat and drought.

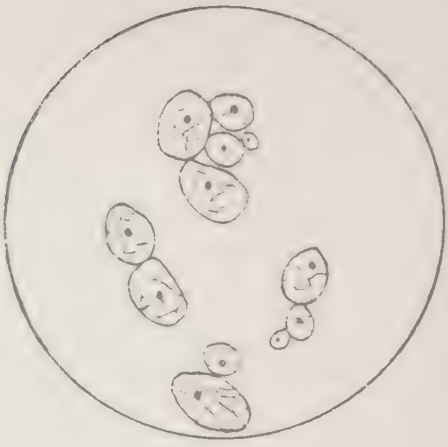
Structure of Yeasts and Moulds.

Yeasts are microscopic unicellular fungi. They are much larger than bacteria and have a definite nucleus. Reproduction is mainly by



Spirilla : curved and spiral cells.
Magnification X 1000.

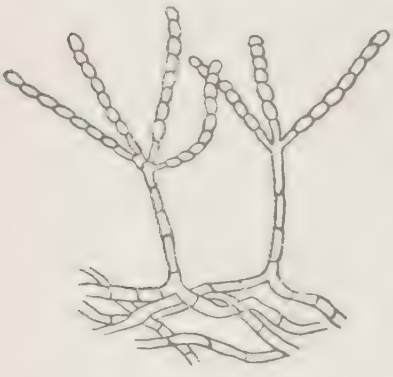
FIG. 36.



Yeast cells showing nuclei and "budding"
Magnification X 1000

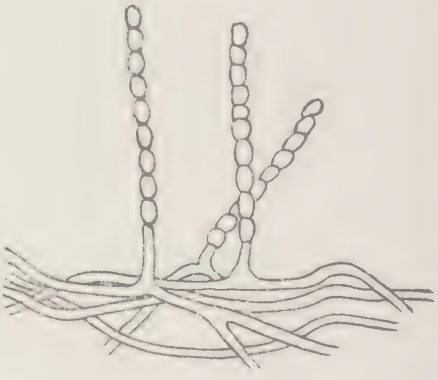
FIG. 37.

" budding " but spores may also be formed. They are not so important in dairying as bacteria, but they do cause certain milk taints and fermentations.



Penicillium sp. showing structure of sporing bodies
Magnified about 400 times

FIG. 38.



Oidium Lactis showing structure of sporing bodies
Magnified about 300 times

FIG. 39.

Moulds are multicellular fungi and consist of filaments often bearing brightly coloured sporing structures. Moulds prefer acid conditions and are important in the ripening of blue-veined cheeses.

Examples of yeasts are *Saccharomyces* and *Torula*, while moulds include *Penicillium*, *Aspergillus*, *Mucor*, *Oidium*, and *Monilia*.

IMPORTANT MICRO-ORGANISMS IN MILK AND MILK PRODUCTS

Bacteria.

Streptococci form chains of varying lengths. The length of the chain is frequently a characteristic of the particular species of streptococcus. Streptococci are the most important micro-organisms in milk and its products. The principal species are :—

S. lactis and *S. cremoris.* These are mainly responsible for the natural souring of milk, the ripening of cream for butter-making and of milk for cheese-making. They are the two predominant species in commercial starters. They can attack the lactose in milk with the production of lactic acid and small amounts of other organic acids. They curdle the milk forming a uniformly smooth and firm curd with a sharp clean odour. No whey separates out and no gas bubbles are produced. These two streptococci grow best at between 70 and 80°F and in the presence of only small quantities of oxygen. They are invariably present in unpasteurised milk and are also widespread in bovine faeces and saliva. They never occur as udder commensals, but gain access to milk from the air and dust of the cowshed, the cow's coat and the utensils. They grow very well on Yeastral milk agar and produce characteristic boat-shaped colonies deep in the medium.

S. lactis maltigenes is very similar to the above in its reactions, but it produces a burnt or malty flavour in dairy produce.

S. liquefaciens grows in short chains and frequently even in pairs of cells. It can acidify and curdle milk, but may also cause "sweet-curdling" and "casein-digestion" together with bitter flavours due to its secretion of proteolytic enzymes. In milk, it is usually overgrown by *S. lactis*, but not at low temperatures. It occurs in the animal intestine and faeces.

S. faecalis is closely related to *S. lactis* but its occurrence is confined mainly to the animal intestine. As it dies rapidly outside the animal body due to the lower temperatures, its presence in a water supply is a sure indication of very recent sewage or faecal contamination.

S. thermophilus can acidify and curdle milk. It is heat-resistant and may not be killed by pasteurisation. It may cause souring in pasteurised milk in sealed containers.

S. citrovorus and *S. paracitrovorus* occur frequently in starters along with *S. lactis* and *S. cremoris* and are thus known as the "associated lactic acid bacteria." They can acidify and curdle milk but produce diacetyl which has an important effect on the flavour of butter.

Lactobacilli are long, slender, rod-shaped bacteria occurring either singly or in chains of varying length. They do not form spores. They acidify and curdle milk producing the same type of curd as the lactic streptococci, but they can produce up to about 4% of lactic acid in the milk whereas lactic streptococci only produce between .8 and 1%. Lactobacilli chiefly occur in faeces, silage and fresh fodder and

gain access to milk from these sources via the dust and air of the cow-shed. Their main importance is in the later stages of ripening of hard-pressed cheese. The commonest species are :—

L. acidophilus which produces up to 4% of lactic acid in milk and grows best at blood heat (37°C).

L. casei only produces 1.5% of lactic acid, but can digest casein slightly. It is widespread in dairy produce and is the most important cheese-ripening lactobacillus.

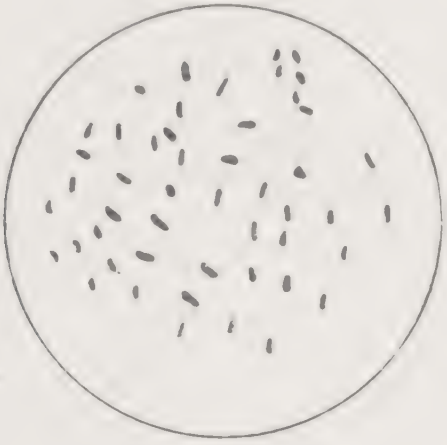
L. thermophilus is found chiefly in pasteurised milk as it grows best at between 50 and 63 C. It can therefore, multiply actively during the " holder " process of pasteurisation.

Lactobacilli . long, slender, curved cells often in pairs and short chains
Magnified about 1000 diameters

FIG.40.

Coliform organisms are small, non-sporing, rod-shaped bacteria with rounded ends. They vary considerably in shape. They occur either as single cells or in short chains. Coliform bacteria can acidify and curdle milk, but the curd produced is soft and contains bubbles of carbon dioxide and hydrogen gases. They are resistant to acid conditions and are therefore, not inhibited in milk by the growth of lactic streptococci. Coliform bacteria constitute a wide group of mainly intestinal types and are themselves only a small section of the much greater *coli-typhoid* group which embraces such pathogenic bacteria as those causing typhoid, paratyphoid, dysentery and bacillary white diarrhoea in chicks. Thus coliform bacteria grow best at body heat (37 C.). Their presence in milk and dairy products indicates faecal contamination and in a water-supply, sewage or faecal contamination. They enter milk chiefly from faeces, flies, silage, fresh fodder and utensils. They cause gassiness and taints of dairy produce. The coliform group of bacteria can conveniently be divided into the

faecal or *Escherichia coli* and the non-faecal or *Aerobacter* types. The former are the commonest intestinal bacteria while the latter less frequently inhabit the intestine, but are widespread mainly in the soil. The *Aerobacter* types grow fairly well on Yeastral milk agar and produce colourless surface colonies. The presence of faecal coliform bacteria in a water-supply indicates recent faecal or sewage contamination. Faecal or "true" coliform bacteria may be distinguished from non-faecal or "atypical" coliform by tests such as the Methyl Red, Koser Citrate and Voges-Proskauer.



Coliform bacteria : small, rod-shaped or coccoid organisms
Magnification X 1000.

FIG. 41.

Proteus and *Pseudomonas* are both putrefactive groups of rod-shaped, non-sporing bacteria. They are found in large numbers in decaying organic matter, soil, faeces and sewage. They do not ferment lactose or acidify milk, but most of them can digest the milk proteins to some extent. These bacteria grow best at blood heat. The most important species are :—

Prot. vulgaris can digest milk proteins almost completely and is thus an active agent in "sweet-curdling" and "casein-digestion" of milk. In the soil, it is one of the most valuable putrefactive bacteria.

P. ichthyosmius can produce trimethylamine from lecithin in milk and its products and so causes a fishy taint.

Pseud. fluorescens, like *P. vulgaris*, can digest milk proteins, but it does not coagulate them. It can thus bring about "casein-digestion" but not "sweet-curdling". It produces a lipase and can split fat, and is one of the agents causing rancidity in butter. It derives its name from its production of a bluish-green fluorescent pigment. It grows best at 22°C.

Pseudomonas fluorescens: rod-shaped non-sporing bacteria
Magnification X 1000.

FIG.42.

P. syncyanae has no action on milk, but if it is growing in milk in association with lactic acid bacteria, it can produce a deep-blue colouration.

Serratia are small, rod-shaped, non-sporing bacteria. There is only one important species, *S. marcescens*, which is also known as *B. prodigiosus*. This organism can acidify milk and produce a soft curd, but it also forms a red pigment which causes red spots on the surface of cheese. It occurs mainly in soil and natural waters.

Micrococci are composed of spherical cells either in pairs or in irregular clusters like bunches of grapes. They do not form spores and many of them are of no importance to milk and its products. The group includes many species, some of which will grow readily on Yeastral milk agar and produce coloured surface colonies like drops of shiny paint. Several of them can acidify milk slightly and can also bring about ropiness or sliminess in milk. One of them, *M. caseolyticus*, can acidify milk and also actively digest the milk proteins. This species may occasionally be a natural inhabitant (commensal) of the udder.

Alcaligenes are small, rod-shaped or coccoid bacteria. They occur singly or in short chains and do not form spores. There is only one important species, *A. viscosus*, also known as *B. lactis viscosus*. This organism is the main cause of ropiness in milk and cream. It is encapsulated when growing in these liquids, that is, the chains of cells surround themselves by gelatinous sheaths. It does not acidify milk but is capable of splitting fat. It occurs in natural waters and in dirty dairies and cowsheds. It usually gains access to milk from contaminated utensils.

Bacilli are relatively large, rod-shaped bacteria, frequently in chains and often actively forming spores. These spores are not larger in diameter than the mother cells, so that the latter are not distorted

A. viscosus, the cause of "ropy" milk showing chains of cells and capsule formation. Magnification X 1000.

FIG. 43.

or swollen. They require large amounts of free oxygen for growth, that is, they are strongly aerobic, and are to be found widespread in soils, dusty fodder and bedding. They are also common in natural waters and decaying organic matter. Several of them such as *B. mycoides* and *B. subtilis*, are troublesome in causing "sweet-curdling" and "casein-digestion" in dairy produce. The digestion of the casein may be so rapid that the initial curdling is not noticed. These bacilli can also produce bitter flavours in milk products. As their spores can easily resist pasteurisation, they can cause bitterness in pasteurised milk, especially at low temperatures such as in cold store. They grow vigorously on Yeastral milk agar and produce grey, spreading surface colonies with a "feathery margin." One very important member of this group is *B. anthracis*, the causal agent of the fatal disease, anthrax.

Clostridia are large rod-shaped bacteria, frequently in chains, becoming swollen in the middle or at one end when forming spores. Unlike the bacilli, they are strictly anaerobic and will not grow in the presence of free oxygen. Clostridia are common in the soil, and often occur naturally in the animal intestine and faeces. Their spores are very resistant and the bacteria grow best at blood heat. Certain species are of importance in dairying :—

C. butyricum is strongly fermentative and rapidly acidifies and curdles milk. It goes on to produce much gas and butyric acid from the lactose. This causes frothiness and an unpleasant taint. The spores survive heat-treatment and so can cause trouble in pasteurised milk.

C. lentoputresens causes slow, sweet curdling and digestion of the milk proteins. It is found both in soil and faeces.

C. botulinum is highly dangerous if present in any food. It is the cause of "botulism" and it produces a powerful poison or "toxin"

which kills by causing paralysis. The spores of this organism are the most resistant of all bacterial spores and can survive for five hours in boiling water, so that they are not usually killed by ordinary cooking. The organism is widely dispersed in soils and may be present on vegetables and fruit and in silage. Dairy produce may very occasionally be contaminated with it, perhaps along with other clostridia such as *C. butyricum*.

Mycobacterium tuberculosis is also known as the " tubercle bacillus " from its habit of forming small lumps (tubercles) in affected tissues. It is the cause of tuberculosis in Man and animals. It is a slender rod-shaped organism, often swollen, and fortunately it does not form spores. It may be present in unpasteurised milk or dairy produce and is derived from cows infected with the disease or from human sufferers coughing over the produce. Such produce is dangerous, particularly to young children. Pasteurisation effectively kills all the tubercle bacilli.

Brucella abortus, the cause of contagious abortion or " brucellosis " in cattle, can also cause " undulant fever " in humans, so called because of the patient's fluctuating temperature. These are small, non-sporing bacteria, usually rod-shaped but they may also be coccoid. Cows suffering from this disease frequently secrete milk teeming with these bacteria.

SUMMARY OF IMPORTANT BACTERIA IN MILK AND MILK PRODUCTS

Species	*Natural Occurrence*	*Importance*	*Mode of Access to Milk*
S. lactis *S. cremoris*	Faeces, saliva, vegetation, silage.	Natural souring of milk. "Starter" bacteria.	Air, dust, cow's coat, silage, utensils, workers' hands.
S. lactis maltigenes	As above.	Produces burnt or malty flavour on souring milk.	As above.
S. liquefaciens	Animal intestine and faeces.	Sweet - curdling casein - digestion, & bitter flavours.	Utensils, dust, cow's coat.
S. faecalis	As above.	Indicates contaminated water supply.	Seldom in milk.
S. thermophilus.	As for S. lactis.	Survives pasteurisation and may sour heat-treated milk.	As for S. lactis.
S. citrovorus *S. paracitrovorus*	As for S. lactis.	"Associated" lactic bacteria : produce diacetyl. Often in starters.	As for S. lactis.
L. acidophilus.	Faeces, silage and in intestine of animals on a milk diet.	Produces 4% lactic acid in milk : assists hard cheese ripening.	Air, dust, silage, and hands of workers handling milk-fed animals.
L. casei.	As above.	Cheese ripening as it digests casein.	As above.

Species.	*Natural Occurrence.*	*Importance.*	*Mode of Access to Milk.*
L. thermophilus.	Mainly in pasteurised milk.	Can survive and multiply during pasteurisation.	Contaminated pasteurising plants.
E. coli.	Faeces mainly.	Acid, gas and bitter flavours in milk, cream and cheese.	Contaminated water, utensils and dirty workers
Aerobacter spp.	Soil, faeces.	As above.	As above.
P. vulgaris.	Soil, faeces, sewage, decayed organic matter.	Sweet - curdling and casein-digestion.	Utensils, dust from fodder, contaminated water, silage.
P. ichthyosmius.	As above.	Produces fishy taints.	As above.
P. fluorescens.	As above.	Casein - digestion and can split fat.	As above.
P. syncyanae.	As above.	Deep blue colour in milk if in association with lactic acid bacteria.	As above.
S. marcesens.	Soil, water-supply	Red spots on cheese.	Contaminated water supply.
M. caseolyticus.	Faeces, soil, dust, air, and perhaps in udder.	Digests casein.	Dust, cow's skin and also perhaps as a commensal in the udder.
A. viscosus.	Water-supply.	Ropy and slimy milk.	Utensils contaminated from the water-supply.
B. subtilis } *B. mycoides.*	Soil, silage, and decayed organic matter.	Sweet - curdling and casein-digestion: bitterness in heat-treated milk.	Dust, cow's skin, contaminated utensils.
C. butyricum.	Soil, intestine, sewage.	Produces butyric acid and gas.	Dust and contaminated utensils.
C. lentoputrescens	Soil, faeces.	Slow curdling of milk and casein-digestion.	Dust, contaminated utensils and cow's coat.
C. botulinum.	Soil, vegetation & fruit.	Poisoning in milk products.	Soil, contaminated water.
M. tuberculosis.	Infected men and animals.	Disease in milk products.	Infected cow, workers coughing.
B. abortus.	Infected cows.	Disease in the milk.	Secreted in milk from infected cows.

YEASTS.

There are only two groups of yeasts, the *Saccharomyces* or " true " yeasts and the *Torula* yeasts. *Saccharomyces* exists as large, spherical cells in chains or clusters; each cell has a definite nucleus. They are very common and widespread in soils, but only a few members of the group can attack lactose and thus become of importance in milk and its products. The species attacking lactose, such as *S. fragilis* and *S. flavo-lactis*, produce acid and gas so causing frothiness in milk and cream. These types are also partly responsible for the fermentation taking place during the production of certain Eastern European milk beverages, such as Yoghourt, Kefir, and Koumiss.

The *Torula* yeasts are of more importance in dairying as most of them can ferment lactose. They consist of smaller, round, oval or elongated cells with nuclei and may exist singly, in chains, or in clusters. They are widely distributed in nature and occur in soils and on vegetation. The types which can ferment lactose produce acid, gas, and alcohols and are the principal agents causing fermentation in the milk beverages mentioned above.

T. cremoris and *T. spherica* produce carbon dioxide from lactose and thus cause gassiness and yeasty flavours in milk and milk products. They are acid-tolerant and can thrive in sour milk and cream.

T. amara can give rise to bitterness in milk products.

T. lactis-condensi is the chief cause of gassiness in condensed milk.

Some *Torula* yeasts cannot ferment lactose, but can split fat. If they occur in large numbers in highly acid butter made from over-ripened cream, they may cause hydrolysis of the fat and so reduce the quality of the butter. They may also produce a red discolouration in such butter.

MOULDS.

Mucor is a " black " mould due to the colour of its sporing bodies. The filaments of the fungus form a delicate branching mass on the surface of the material upon which the mould is growing. *M. mucedo* is the commonest species and it can digest milk proteins and split fat and so cause faults in butter and cheese.

Penicillium is a " green " mould as its sporing bodies are blue-green in colour. It grows best under moist, acid conditions, as for instance on soft cheese. There are several species of importance in dairying :—

P. glaucum and *P. roquforti* can digest casein and constitute the " blue veins " of Stilton, Gorgonzola and Roquefort cheese. The products of the moulds' growth gives these cheeses their characteristic flavour. They occur naturally on vegetation and in silage and their spores are fairly resistant to heat and drought.

P. camemberti is similar to the above and is partly responsible for the ripening of soft cheeses such as Camembert. It can digest casein but cannot coagulate it.

P. brevicaula can produce a " turnipy " taint in milk and its products.

P. casei produces a reddish-brown discolouration of cheese-rinds.

P. notatum is the most important member of this group as this is the species of mould from which the invaluable drug, *penicillin*, is extracted.

Cladosporium is a dark, olive-black coloured mould. Several of its species can discolour soft cheese as for instance, *C. herbarum*, while others such as *C. butyri*, can produce fruity taints in milk and are often present in rancid butter.

Oidium lactis commonly occurs on vegetation and is usually present in sour milk, soft cheeses and butter. It is colourless, grows well under acid conditions and can ferment lactic acid. It is valuable in ripening cheeses such as Camembert and Gorgonzola and gives them their flavour, but it can also cause rancidity in butter.

Growth of Bacteria.

Under optimum conditions, bacterial growth is stupendous. Each cell will duplicate itself in 20-30 minutes. If each cell divides every 20 minutes and no cells die, one cell in 12 hours will produce 70,000,000,000 (70 thousand million) bacteria. In 24 hours, it will produce 500 tons of bacterial cells. Fortunately, this rapid rate of growth is soon arrested, usually by the accumulation of waste products of the bacteria themselves. Thus in the souring of milk, the concentration of lactic acid eventually kills off the lactic streptococci.

The growth of bacteria is influenced by :—

(1) The food supply, known as the " substrate."

(2) The moisture supply. Bacteria cannot grow under dry conditions.

(3) The air supply. Most bacteria require free oxygen in order to live.

(4) The presence of light, especially sunlight which kills off many disease-producing bacteria as, for example, the tubercle bacillus.

(5) The temperature. All bacteria have an optimum temperature for growth and their activity is less above and below this temperature.

(6) The chemical and physical environment. Certain substances, known as disinfectants, and certain conditions, such as drought, are directly harmful to bacteria.

In the dairying industry, harmful bacteria are controlled mainly by these last two factors. The temperature factor is utilised in the cooling and pasteurisation of milk and in the steam-sterilisation of equipment, while the use of detergent chemicals such as hypochlorite solutions, is based on the sixth factor.

Nearly all the changes which take place in milk and affect its taste, odour and appearance after coming from the cow, are due to the actions of bacteria. Methods of handling milk and its products are directed largely towards the control of these bacteria.

SOURCES OF BACTERIA IN MILK

Milk in the alveoli of the udder of a healthy cow should be sterile. On its passage through the milk ducts and teat canals, however, it usually picks up various bacteria. These are known as " commensals " as they are natural inhabitants of these parts of the udder. In a healthy cow, they are all harmless, but in the udder of a diseased cow, disease-producing (pathogenic) bacteria such as those of mastitis and contageous abortion, may also be found. Freshly drawn milk may contain up to 500 bacteria per millilitre. Fore-milk always contains the most bacteria and should be discarded, but strippings also have a high bacterial count due to the excessive handling of the teats during that operation. If the cow is not fully milked out, bacteria may develop in the residual milk in the ducts and teat canals, so that at the next milking, the cow may produce milk containing an unduly large number of these organisms. In normal clean milk from a healthy cow the few bacteria initially present are mainly harmless micrococci and staphylococci. These grow best at blood heat (37 C) and are inactivated when the milk is cooled.

Once drawn, however, milk is open to contamination and dirty milk may contain millions of bacteria per millilitre. If it is cleanly produced and efficiently cooled, it should not contain more than about 6,000 bacteria per millilitre. The sources of bacterial contamination of milk include the following :—

(1) *Dirty utensils* are the main sources. The milking-machine is a frequent offender as it is difficult to sterilise properly. Coolers are also hard to clean satisfactorily due to their corners and corrugated surfaces. Muslin strainers over coolers and churns often do more harm than good. Single service cotton filter mediums should always be used. As these are used once only and then discarded, they cannot continue to contaminate the milk as muslins frequently do when dirty. After washing and sterilising, utensils should be left in a dust-free atmosphere to cool and dry. Utensils may be tested for bacterial contamination by swabbing them with sterile swabs or rinsing with sterile water or Ringer's solution, and then counting the number of bacteria in the swabs or rinsings. The bacteria usually obtained from utensils consist mainly of coliform types, sporing bacilli which may " seed " on to metal surfaces, and organisms such as *Streptococcus lactis* which cause souring, and those responsible for bad flavours.

(2) The *water supply* is second only to utensils as a source of contamination. It must be clean and should be tested periodically for disease and sewage contamination, and also for the numbers of bacteria in it.

(3) The *atmosphere* in the cowshed may contain many bacteria in dust if hay or straw has been shaken about, and this dust often contaminates the milk. Foddering, cleaning out and bedding down should be done only after milking.

(4) The *cow's coat* is a serious source of contamination if it is fouled with dried dung, which is full of coliform bacteria, or if it is dusty from bedding which is a source of sporing micro-organisms.

The use of hypochlorite detergent in the wash-water will kill the bacteria on the skin of the cow's udder and flanks.

(5) The *milking personnel* may cause contamination and should have clean hands and clothes. Persons with sore throats or infectious diseases should not handle milk. Coughing can infect the milk with pathogenic bacteria.

Results of Bacterial Growth in Milk.

Bacteria when growing produce enzymes which bring about the following chemical changes in the milk constituents :—

(*a*) The lactose or milk sugar is fermented into organic acids such as lactic, acetic and butyric and also carbon dioxide. Lactic acid curdles the milk and causes souring while acetic and butyric acids produce bad flavours and carbon dioxide causes gassiness.

(*b*) The casein may be split up into amino-acids due to "sweet-curdling" and "casein-digesting" enzymes. This can cause sliminess and bitterness.

(*c*) The butter-fat may be split up into its component free fatty acids and glycerol, and produce rancidity, especially in butter.

Any bacteria existing in long chains—such as streptococci—or in capsules will cause respectively ropy and slimy milk. The speed of the above enzymic changes depends mainly on the number of appropriate bacteria in the milk, but also on the temperature.

THE PRODUCTION OF CLEAN MILK

Clean milk production aims at eliminating all sources of contamination with dirt and bacteria, and so reducing to a minimum the numbers of bacteria in the milk. Milk which has been cleanly produced always has a high keeping quality. Utensils not sterilized form by far the most serious source of bacteria in milk, while lack of thorough and quick cooling, together with failure to hold the milk afterwards at a sufficiently low temperature, are other causes of poor keeping quality.

In the cowshed, the cleanliness of the cow and of the milking personnel and the purity of the cowshed atmosphere determine the amount of the initial contamination. It has been proved by experiment that cleaning and clipping the udder and flanks of a cow may reduce this by 80%. Milking should be quick and thorough and cows with udder diseases such as mastitis should be milked last and their milk excluded from the herd bulk. The use of hooded buckets in hand-milking may reduce contamination considerably. Milking stools should be kept clean and preferably should be of metal construction. This allows them to be easily steam-sterilised. As the stool is the last object the milker touches before he starts to milk the cow, its cleanliness is most important.

The actual number of bacteria which contaminate milk from utensils varies considerably. In a recent test with a milk cooler on a commercial dairy farm, the importance of the utensils was strikingly

shown. After rinsing with 1,500 millilitres of sterile water, the rinsings were found to contain more than 16,000 bacteria per ml. This meant that the whole 1,500 ml. of rinsings contained at least 23 million bacteria, all of which were clinging to the surfaces of the cooler and which would have gone into the first batch of milk to be cooled. The use of utensils as grossly contaminated as in this instance can only mean the production of milk of very inferior keeping quality. After efficient sterilisation, the increase in bacterial content due to the utensils should not be more than five bacteria per ml. in milk 12 hours old. Sterilisation with steam or approved hypochlorite solutions is the principal factor in maintaining sterile utensils; scalding with boiling water is not nearly sufficient. Steam and hypochlorites are however, only effective as sterilising agents if the surfaces to be sterilised are free from dirt and traces of milk solids, which protect the bacterial cells from destruction.

The normal routine when sterilising utensils is first to wash them in *cold* water before the milk has dried on. This removes most of the milk solids except the fat. The use of hot water first would tend to coagulate the milk proteins and make them difficult to remove. The utensils are then scrubbed in hot water containing a detergent such as washing soda, which removes the fat, and finally, after a copious rinse in hot water to remove all traces of the detergent, the utensils are subjected to live steam at a temperature of at least 210°F for 10 to 15 minutes. This should be done preferably in a steam chest, which should be large enough to hold all the utensils and stools and which should be fitted with a steam coil to dry them after steaming. Milk churns may be steamed inverted on a churn steaming stool. As the steam issuing from the steam jets on these stools may only have a temperature of between 89° to 105° C, it is necessary to steam for at least three minutes. In practice, this full steaming period is seldom given and the sterilisation of churns on these stools is not always satisfactory. In place of steam, utensils can be sterilised quite satisfactorily by the use of approved hypochlorite solutions. These are normally used at a working strength of about 200 parts per million of available chlorine (2½ oz. or 2 eggcupfuls of stock solution per 2 gallons of clean water) and the utensils are placed in contact with this solution for at least 20 minutes.

The commonest source of poor keeping quality in market milk is contamination from the milking machine. It is estimated that in 95% of milks with very high numbers of souring bacteria, the cause is inefficient sterilisation of the milking machine units. A milking machine if properly used and carefully cleaned and sterilised at all times, will give milk comparable in keeping quality to the best hand milking, but careless handling of the machine coupled with failure to sterilise it properly, is the surest way to produce milk of low keeping quality. The best way to clean and sterilise a milking machine unit is, first to suck a bucketful of cold or warm water through the teat-cups and milk tubes before dismantling. The teat-cup cluster should be lifted clear of the water several times to suck in air. This provides an "air-brush" in the teat-cups and gives a more effective rinse than a straight flow of water. The teat-cups should then be scrubbed inside and out

with a mild, warm detergent solution, as also the outside of any soiled tubes. Following this, a bucketful of strong detergent solution as near boiling point as possible, should be sucked through the unit without breaking the flow. The final sterilisation may be carried out in several ways. A bucketful of boiling water may be sucked through the unit without breaking the flow; steam from a jet may be blown through the tubes and teat-cups for at least three minutes; or the unit may be treated with a standard hypochlorite solution for about 20 minutes.

The milking machine vacuum pipe-line can, if neglected, prove a troublesome source of bacteria in the milk. Some of the taps should be left open between milkings to ventilate the line and it should be disinfected once a week. This may be done by sucking a bucketful of warm hypochlorite solution through the tap farthest from the vacuum pump. The detergent will pass through the pipes and disinfect them. It will collect in the sanitary trap but care should be taken that this does not overflow, and liquid be sucked into the pump itself. If a unit bucket becomes too full of milk, say from a heavy yielder, then, if the operator is careless, milk will be sucked up the vacuum tube into the pipe-line and will stay there. To prevent such milk souring and being a source of contamination, the pipe-line should be drained through the drainage cock every day. This will also remove any condensed moisture which has accumulated there.

In the case of modern auto-recorder and recorder-releaser plants in milking parlours, all the parts including the teat-cups may be sterilised *in situ* by steam under pressure, as the whole system is a closed circuit. The makers' instructions for sterilisation must be followed closely otherwise the efficiency of sterilisation will not be satisfactory. Some dairy farmers, although sterilising their recorders twice daily without dismantling, also strip the whole plant periodically and thoroughly scrub it with a detergent solution. Provided the steam sterilisation is carried out properly, this is not necessary.

As soon as possible after it is drawn from the cow, the milk should be removed from the cowshed and filtered or strained. Filtering has no beneficial effect on the keeping quality or the flavour of the milk as it simply removes large particles of dirt. No filter will remove bacteria. Only single service cotton filter mediums should be used. These are used once and then discarded. Muslin cloths used regularly are worse than useless, as these are seldom effectively sterilised and simply cause contamination.

The cooling of the milk should be thorough and efficient. It should be carried out as soon as possible in order to inhibit the growth of any bacteria present. The cooler should be of a design which is easy to clean and it must be sterilised regularly. If possible, it should be of stainless steel, but copper is quite satisfactory if it is properly tinned over. Otherwise the warm milk dissolves traces of copper which may later lead to the development of tallowy flavours in the milk or milk products. The flow of milk and water in the cooler should be so regulated that the temperature of the milk is reduced to within 2° of that of the water. About four gallons of water are generally required to cool one gallon of milk from 95° F down to 60° F.

Small mechanical refrigerating units which circulate chilled water or brine through the cooler are excellent. Usually only the lower half of the cooler is connected to the refrigerator. In the top half, water is circulated. The cost of these units is justified only on those dairy farms with a large milk output. These refrigerators will cool the milk to below 40°F even in the warmest summer weather and may also provide sufficient refrigeration for a small cold-store in the dairy.

The modern idea of " immersion cooling " where the churns full of the fresh warm milk are placed in a bath of chilled water and left until collected is admirable as it combines cooling and cold storage in one operation. Moreover, it dispenses with the risk of contamination when the milk flows over an open cooler. Immersion cooling is still undergoing development however, and at the moment is too expensive for the average dairy farm, but on very large farms its installation would be well justified.

After cooling, the milk should be kept cool until collected. If milk churns are allowed to stand in the sun and the milk gradually becomes warm again, the beneficial effect of cooling is completely nullified and the bacteria in the milk will commence to grow vigorously so lowering the keeping quality. The dairy is generally the best place to store milk as it is cool and clean. If the churns must be left at the farm road-end until collected by lorry, then a simple churn shelter should be constructed.

Clean milk production consists essentially of two parts. First the numbers of bacteria gaining access to the milk is kept as low as possible and secondly, the growth and multiplication of those bacteria which have succeeded in entering the milk are checked. The points of importance to be observed in producing clean milk are summarised as follows :—

(A) *Prevention of contamination of the milk with bacteria.*

(1) The cowshed should be kept clean and should be swilled out regularly with plenty of clean water. The ventilation should be adequate to preserve a clean sweet atmosphere free from dust.

(2) The cows should be kept well groomed and clipped. Before milking, their udders should be washed with a warm, mild, hypochlorite solution.

(3) The milkers should be clean in person and should wear clean overalls. Persons suffering from infectious diseases or from sore throats and coughs should not be allowed to handle milk.

(4) Milking stools should be kept clean and should be sterilised regularly.

(5) All utensils, milking machine units, and strip-cups should be sterilised at least once daily and preferably twice. If they are sterilised only once, such as after the morning milking, they should be rinsed copiously with cold water after the evening milking before the milk has had time to dry on to them. They should be placed to drain overnight in a cool, clean room. Coolers should be treated similarly and should be dismantled daily for sterilisation. Milking machines

should be stripped down and thoroughly cleaned at least once a week and preferably twice.

(6) The vacuum pipe-line of the milking machine should be left open to ventilate between milkings and any moisture or milk in it should be drawn off daily through the drainage cock. The pipe-line should be disinfected once a week by sucking warm, mild detergent solution through it.

(7) Milking should be carried out as rapidly as possible. Milking buckets should be hooded. Teat-cups should not be allowed to touch the floor or to suck in air.

(8) The milk should be removed from the cowshed as soon as possible after it is drawn and, particularly, should never be left exposed to the atmosphere in uncovered containers.

(B) *Prevention of the growth and multiplication of bacteria already in the milk.*

(1) The milk should be cooled as soon as possible, preferably to below 50° F. The cooler should be covered and should be placed in a clean, cool room. Persons should not cough near it.

(2) After cooling, the milk in the churns should be kept cool and never allowed to stand in the sun or near hot objects.

If any one of the above points is neglected and the milk becomes dirty and of low keeping quality, not only is the producer himself penalised, but the consumer also is affected. Milk of poor keeping quality causes trouble right throughout the various processes of manufacture such as butter-making, cheese-making, condensing and drying. Moreover, pasteurisation is not nearly so effective if the milk contains large numbers of bacteria, especially if these are of heat-resistant types. Thus the whole efficiency of the dairy industry is dependent on the care and diligence of the dairy farmer.

NATIONAL MILK TESTING AND ADVISORY SERVICE

This scheme was introduced in June, 1942, with the following objects in view :—

(1) To detect milk supplies of inferior keeping quality and to assist the producers of such milk to improve their standards of production.

(2) To minimise losses of milk through souring by the exclusion of unsatisfactory milk supplies from bulk milk.

(3) To set up a service to advise on problems of producing high quality milk.

(4) To advise creameries and dairies on the best methods of handling and processing milk.

The whole scheme is based on the regular testing of milk supplied by individual producers. Tests are carried out at the point of delivery to the creamery or dairy. This permits of the detection and tracing

of supplies of poor keeping quality. It also enables the source of contamination to be determined and investigated by the advisory officers. Any consignment of milks, including Designated milks, may be subjected to test. The ultimate intention is to test all milk supplies twice monthly by the standard method and to subject churns of milk of doubtful quality to the " Platform Rejection Test " on arrival at the creamery.

The " Standatd Routine Test " is the Temperature Compensated Resazurin Test described in a later section. The milk is graded into Categories A, B or C according to the comparator disc reading obtained. A producer whose milk falls into Category C at a routine test is notified of the fact and an advisory visit made to the farm to investigate the source of the trouble. In winter, milks at the lower end of Category B, that is with a disc reading of $2\frac{1}{2}$-1, are treated similarly because in summer these would have probably dropped to Category C. The " Platform Rejection Test " is the 10-minute Rezasurin Test described later. The milk from churns of doubtful quality is graded into specific classes. If the disc number is above 4, the milk is A (*market* standard). Milk with a disc number of $3\frac{1}{2}$-1 is B (*manufacturing* standard) while milk with a disc number below $\frac{1}{2}$ is C (*rejection* standard). Milk graded C in this test is of very poor keeping quality and is returned to the producer. Even a small amount of such milk in a larger bulk of better quality milk is liable to cause souring of the whole consignment. Milk of grade B is suited for consumption as liquid milk within short distances of the creamery, but if it has to be transported over long distances in bulk, it is liable to go sour. If facilities for manufacture are at hand, this milk is salvaged but otherwise it is returned to the producer. Milk may be rejected irrespective of the results of the platform test due to a food taint or absorbed flavour.

Provision is also made in the Scheme for the regular testing of milk churns for the presence of bacteria. Dirty churns are often responsible for milk of poor keeping quality. By regular testing, creameries continually returning dirty churns to the farmer can be detected. The churns are graded " satisfactory," " fairly satisfactory " or " unsatisfactory " according to the numbers of bacteria they contain. Where necessary, visits are made to the creamery and advice is offered on the best technique for sterilising churns.

Where dairy farmers are found to be continually producing milk of low keeping quality and are making no endeavour to improve their methods of production, action outside the Scheme may be taken. Details of the results of the tests may be sent to the local sanitary authorities who can take action in accordance with the Milk and Dairies Orders. Licensing authorities are notified of supplies of Designated milks which are graded Category C in the twice monthly routine test or which are rejected on the platform as having a disc reading of $3\frac{1}{2}$ or less. If the milk is persistently of this low quality, the licence for the production of this particular grade of Designated milk may be withdrawn.

LEGISLATION RELATING TO THE PRODUCTION OF MILK

There is a comprehensive mass of Acts, Orders and Regulations governing the various aspects of milk production. Without this legislation, the marketing of milk would be almost chaotic and the quality and cleanliness of the milk supply would be very variable indeed and even dangerous. These laws and regulations promote the orderly marketing of milk and safeguard public health by controlling the conditions under which the milk is produced and sold. The principal milk regulations affecting the dairy farmer are summarised below :—

(1) All persons keeping cows and all premises wherein cows are kept must be registered by the local authority on behalf of the Ministry of Health. They must also be licensed by the Ministry of Food and the Milk Marketing Board. The only exemption is in the case of those producers who retail milk to their staff for consumption in the latters' households, but who do not otherwise sell it. Persons may not sell milk from a farm on which milk has not previously been produced without the consent of the County Agricultural Executive Committee.

(2) Persons may sell milk wholesale only to the Milk Marketing Board and only under the latter's conditions and terms.

(3) The Milk Marketing Board prescribes the conditions under which producers may sell their milk with regard to description, price, buyer, etc. and also lays down directions as to the grading, packing and transportation of the milk.

(4) Under the Use of Milk (Restrictions) Order, 1945, the use of liquid milk for the making of table cream and ice-cream is, with certain exceptions, prohibited. It is, however, still permissible to use skimmed milk powders for ice-cream manufacture.

(5) Milk offered for sale must have been produced on registered premises and it must be the product of cows which have been calved for more than three days.

(6) Before milk may be sold, the local authority must approve the quality of the buildings wherein it is produced, with regard to the form of construction, type of floor (which must allow liquid matter to be removed easily and which must be channelled to minimise soiling of the cows) and amount of floor space available per cow, type and efficiency of the ventilation system, lighting (both artificial and daylight), quality and quantity of the water-supply and the means of disposal of drainage.

(7) Milkers must keep their persons and clothing clean and milking must be carried out in a good light. The flanks, udders and teats of the cows must be wiped with a clean damp cloth prior to milking to remove dirt. Milkers' hands must be washed and dried before milking.

(8) Under the Food and Drugs Act, 1938, it is a punishable offence to sell or offer or expose for sale for direct human consumption

or for manufacture into milk products for human consumption, the milk from any cow suffering from :—

(*a*) tuberculosis with emaciation.
(*b*) tuberculosis of the udder.
(*c*) acute mastitis.
(*d*) anthrax.
(*e*) foot-and-mouth disease.
(*f*) actinomycosis of the udder.
(*g*) suppurating sores on the teats.
(*h*) septic condition of the uterus.
(*i*) comatose condition.
(*j*) any condition of the udder or teats likely to contaminate the milk,

unless the farmer can prove he had no reason to suspect the presence of any of these conditions among his cows. The farmer is considered to have had reason to suspect any of them if, with ordinary care, he could have detected it.

(9) Milk producers must notify local authorities of the incidence of certain diseases among their cows and also any infectious diseases among their workers who handle the milk. The local Medical Officer may demand a list of the producer's customers in the event of an outbreak of milk-borne disease in a community. Such a list proves very useful in tracing the source of the disease outbreak.

(10) Under the Tuberculosis Order, 1938, the milk from any cow suffering from chronic udder disease, tubercular emaciation or chronic cough must be excluded from the herd bulk milk until a veterinary surgeon has examined the cow. Such milk must not be mixed with bulk milk until after six weeks have expired to permit microscopic and biological tests to be carried out if necessary or until the farmer is officially informed that such tests are not necessary. Any milk from a cow suffering from one of these conditions must be effectively boiled or sterilised. The utensils used for such milk must also be thoroughly sterilised before they are used for other milk.

(11) Regulations are in force controlling the cooling of milk. Milk must be cooled as soon as possible to at least within 5° of the cooling water, unless the milk is delivered at once to a dairy with facilities for cooling it to below 55°F. Milk must not be stored in cowsheds, dwelling houses or in any place where it may be exposed to impure air and vessels containing milk must be properly covered to exclude dust, dirt and flies. There are, however, no laws in operation prohibiting milk from being stored near volatile substances which may taint it.

(12) Milk churns and other containers should be sealed for transport and the transfer of milk from one container to another is permitted only in registered premises.

(13) The use of milk containers, including milk bottles, belonging to another party is illegal unless that party's consent is previously obtained. Wooden containers may be used only for butter-milk.

(14) All utensils and appliances used in any way so that they come in contact with milk must be reserved for milk only. They should be kept in a state of complete cleanliness at all times and should only be stored in a clean cool place free from dust or dirt.

(15) While steam sterilisation of utensils is preferable, measures have been introduced during the late war permitting the use of certain approved sodium hypochlorite solutions for the purposes of sterilisation.

(16) All milk offered for sale must be genuine and unadulterated. The addition of thickening or colouring matter or of any form of preservative is forbidden. Similarly, the addition or abstraction of any form of milk solids is prohibited.

(17) In the event of a sample of milk being taken for chemical analysis and being found to contain less than 3% of butter-fat and/or less than 8.5% of solids-not-fat, the milk shall be considered as not genuine until the contrary is proved. These figures are known as the "Presumptive Legal Standards." For separated milk, the presumptive legal standard is 8.7% solids-not-fat. In the case of prosecution, the onus of proving that milk below these standards is genuine falls on the producer. The procedure for this is known as the "Appeal to the Cow" and consists of taking a sample from the cow in the presence of an inspector. If this sample proves to be below the legal standards, the case against the farmer is dismissed.

(18) Farmers and dairymen must keep records of their milk production and must submit returns regularly. They may retail milk only to registered customers and may obtain milk only from those sources officially nominated.

All these rules and laws are enforceable and anyone breaching them is liable to be punished, ignorance of the law not being accepted as an excuse.

GRADED MILKS

Under the Milk (Special Designations) Order, milks of a high quality of cleanliness and others which have undergone special treatment may be designated in various grades. These graded milks command a premium. Grades of milk now in operation in England and Wales are *Tuberculin Tested*, *Tuberculin Tested* (*Certified*), *Tuberculin Tested* (*Pasteurised*), *Accredited* and *Pasteurised*. These special designations may only be applied to the milk if the seller holds a licence for the production of that particular grade and if the conditions of the licence are complied with. In the case of the English and Welsh grades, the conditions under which each grade may be produced and sold and the quality tests which it has to pass are as follows:—

Tuberculin Tested. This grade of milk can only be produced from cows which are tested for tuberculosis at regular intervals, as under the Attested Herds Scheme. The cows must also be clinically examined for general health by a veterinary surgeon at least once every six months. The milk must be delivered to the consumers in containers under

specified conditions. If the milk is actually bottled on the farm, it may be designated as *Tuberculin Tested* (*Certified*). Cleanliness tests for both these grades of milk include the standard methylene blue test where the milk should not decolourise the dye in $4\frac{1}{2}$ hours in summer or $5\frac{1}{2}$ hours in winter, and the presumptive coliform test where the milk should not contain coliform bacteria in 1/100th ml. These tests are carried out on routine samples drawn on the farm at regular intervals by the local authorities.

Accredited milk is produced under the same conditions as the previous grades except that the cows are not subjected to the tuberculin test and, therefore, are not necessarily free from tuberculosis. If the milk is bottled on the farm, it may be further designated as " Farm Bottled."

The following conditions must also be observed in the case of these three special designations :—

(*a*) Each grade must be kept separate from all other milk except where it is contained in sealed vessels.

(*b*) Before being used for one of these grades, all containers and apparatus which have at any previous time been used for other milks, must be thoroughly washed and sterilised.

(*c*) No milk of these grades may be in a dairy in which there is also other milk unless it is in sealed containers.

Tuberculin Tested (*Pasteurised*) milk is produced from cows tuberculin tested and clinically examined as for " Tuberculin Tested " milk. The milk is subjected to one of the officially approved pasteurisation processes, that is, either the " Holder " method or the more modern " High-Temperature Short-Time " method. The conditions of sale and of delivery to consumers are the same except that the word " Pasteurised " must be added to " Tuberculin Tested " on the containers. There is no coliform test prescribed for this grade, but in the appropriate methylene blue test as described later, the milk should not decolourise within half-an-hour of the addition of the dye. The milk should not contain more than 30,000 bacteria per ml. There is no legal phosphatase test stipulated, but all milk which has been heat-treated should not give a result of over 2.3 Lovibond Blue units in the standard phosphatase test.

Pasteurised milk has to pass laboratory tests similar to those of the previous grade, but there are no conditions of health in the herd stipulated. The word " Pasteurised " on a suitable label must be affixed to every vessel in which the milk is transported or exposed or offered for sale.

In Scotland, the grading of Designated milks and the conditions under which they are produced and sold are rather different. Graded milks in Scotland include *Certified*, *Tuberculin Tested*, *Tuberculin Tested* (*Pasteurised*), *Standard*, *Pasteurised* and *Heat-Treated*. The conditions under which these grades may be produced in Scotland are as follows :

Certified milk may only be produced from cows which are clinically examined thrice yearly at intervals of 3-5 months and which are sub-

mitted to regular tuberculin tests as under the Attested Herds Scheme. The milk must be cooled to at least 50 F as soon as it is produced and must be bottled on the premises into steam-sterilised bottles not more than one quart in capacity. These must be capped with an overlapping cap marked " Certified " with the name of the farm and the day of production. The milk must not contain coliform bacteria in 1/10th ml. and must have not more than 30,000 bacteria per ml.

Tuberculin Tested milk must be the produce of cows which have been regularly tested for tuberculosis and general health as for " Certified " milk. This grade of milk need only be cooled to 60 F and should not contain coliform bacteria in 1 100th ml. while the maximum numbers of bacteria per ml. permitted in such milk is 100,000. It must be despatched in sealed, sterilised cans labelled and marked with the farm address and the bottling premises, although it may be bottled on the farm. The words " Tuberculin Tested " must appear on each bottle cap.

Standard milk corresponds to the *Accredited* grade of milk in England and Wales. The cows must be clinically examined thrice yearly at intervals of 3-5 months. There is no tuberculin test, but the herd must not contain any animals which, to the knowledge of the producer, have reacted to the tuberculin test before introduction to the herd. The laboratory tests for bacteria are the same as for " Tuberculin Tested " milk as also are the conditions of delivery. The bottle caps must bear the word " Standard." This grade may also be bottled on the farm.

Tuberculin Tested (*Pasteurised*) milk may be produced under the same conditions of animal health as the " Tuberculin Tested " grade. It has to be cooled immediately after heat-treatment to 50 F or lower. Coliform bacteria should be absent in 1 10th ml. and there should not be more than 30,000 bacteria per ml. Conditions of delivery and bottling are the same as for " Tuberculin Tested " milk but the word " Pasteurised " must be added to the words " Tuberculin Tested."

Pasteurised milk in Scotland is similar to the *Pasteurised* grade in England and Wales and all the containers in which the milk is transported or sold must be labelled " Pasteurised." Such milk must satisfy a phosphatase test of 2.3 Lovibond Blue units and coliform bacteria should be absent in 1 100th ml. In certain areas, there is a methylene blue test in use and this grade of milk should not decolourise the dye by noon on the day after the sample was taken. This particular test must be started within one hour of the sample being received at the laboratory and must be carried out at atmospheric shade temperature.

Heat-treated milk must be sold and transported in vessels marked or labelled " Heat-treated." It is a form of sterilised milk and should contain few bacteria. The only prescribed laboratory standards are that the milk, in the phosphatase test, should not give a result of over 2.3 Lovibond Blue units and that in the appropriate methylene blue test carried out at atmospheric shade temperature, the dye should not lose its colour before noon on the day after the sample was taken.

SUMMARY OF REGULATIONS AND STANDARDS FOR DESIGNATED MILKS

Designation.	*Conditions of Animal Health.*	*Laboratory Test Standards.*	*Regulations for Sale and Delivery.*
England & Wales. Tuberculin Tested	Regularly tested for tuberculosis and clinically examined for general health.	Must not decolourise methylene blue in 4½ hours in summer or 5½ hours in winter : coliform to be absent in 1/100th ml.	Containers or bottles to be of less than 2 gallons in capacity and to bear name and address of bottling plant with day of production and the words "Tuberculin Tested" on the cap.
Tuberculin Tested (Certified)	As above.	As above.	As above but is bottled on the farm and the word "Certified" added to "Tuberculin Tested."
Accredited.	Clinically examined for general health twice yearly.	As above.	As for "Tuberculin Tested" but if farm-bottled, the words" Farm Bottled" may be added to the word "Accredited."
Tuberculin Tested (Pasteurised)	Tested and examined as for "Tuberculin Tested" grade.	Must not decolourise methylene blue in ½ hour : not more than 30,000 bacteria per ml. : phosphatase test of 2.3 Lovibond Blue units or less.	As for "Tuberculin Tested" except that the word "Pasteurised" must be added.
Pasteurised.	No stipulated conditions.	Must not decolourise methylene blue in ½ hour : not more than 100,000 bacteria per ml. : phosphatase test of 2.3 Loviband Blue units or less.	All containers in which the milk is transported or sold to bear the word "Pasteurised."
Scotland. Certified.	Regularly tuberculin tested and clinically examined for general health thrice yearly	Not more than 30,000 bacteria per ml. : no coliform in 1/10th ml.	Cooled to 50°F or lower and bottled on the farm with the word" Certified" on each bottle cap along with name of the farm and the day of production.

Scotland. Designation.	*Conditions of Animal Health.*	*Laboratory Test Standards.*	*Regulations for Sale and Delivery.*
Tuberculin Tested.	As above.	Not more than 100,000 bacteria per ml. : no coliform in 1/100th ml.	Cooled to 60 F or lower, otherwise as for the English "Tuberculin Tested" grade.
Standard.	As above but no tuberculin test.	As above.	As above except that the word "Standard" replaces "Tuberculin Tested" ; may be farm bottled.
Tuberculin Tested (Pasteurised)	As for "Tuberculin Tested."	Not more than 30,000 bacteria per ml. : coliform absent in 1/10th ml. : phosphatase test of 2.3 Lovibond Blue units or less.	As for "Tuberculin Tested" but word "Pasteurised" to be added. Cooled to 50°F or lower.
Pasteurised.	No conditions stipulated.	Coliform absent in 1/100th ml. : phosphatase test of 2.3 Lovibond Blue units or less. In some areas, must not reduce methylene blue by noon next day.	As for the English "Pasteurised" grade.
Heat-treated.	As above.	Phosphatase test of 2.3 Lovibond Blue units or less: must not decolourise methylene blue by noon of next day.	All containers in which the milk is transported or sold to be labelled "Heat-Treated."

THE WATER SUPPLY TO THE DAIRY FARM

The water supply is of the first importance on a dairy farm. It must be chemically pure and should contain as few bacteria as possible. It should be plentiful without great seasonal fluctuations. Water is the last substance to come in contact with the surface of the utensils before they are used for milk, and also to touch the skin of the teats before they become wet with milk when the milking machine is applied. If then, the water supply is polluted and the polluted water used to wash the utensils and the udders and teats of the cows, the milk is most likely to become contaiminated. This invariably leads to deterioration in the milk, such as ropiness or sliminess and to objectionable taints. There are numerous cases on record of outbreaks of these faults being traced through the utensils to a contaminated water supply. Moreover, cows obliged to drink polluted water from a stream or a

pond may develop digestive disorders which can have the effect of temporarily lowering the yield of milk or which can cause the animal to secrete tainted milk. It is no exaggeration to state that the purity of the water supply to a dairy farm or a creamery should be fully as high as the purity of a domestic supply to a community for direct human consumption.

Most natural waters contain bacteria and the chief factor limiting their numbers is the supply of food for them, although temperature also exerts a strong controlling influence. As the main food supply for bacteria in water is organic matter, it follows that those water supplies with a high organic content are liable to contain very large numbers of bacteria. The exception to this is in the case of water supplies coming from peaty land. These may be rich in organic matter and indeed are frequently slightly discoloured by it, but they are usually safe and contain very few bacteria.

The main sources of organic matter in a water supply are sewage and surface drainage. This means that water supplies drawn from streams or lakes near large towns or flowing through intensively cultivated arable land are liable to be polluted to some degree and should not be used in connection with milk production. Spring water and deep well water are usually very pure and may be used safely in dairies, but in the case of farms without modern sanitation, well water can become contaminated with sewage. Care must be taken to see that liquid sewage does not seep through the walls of the well and so contaminate the water. Such contamination was the cause of many fever epidemics in the 17th and 18th centuries. Wells liable to be contaminated in this manner should always be situated a good distance away from any source of sewage and, if possible, uphill from it to avoid the risk of contamination by gravitation.

The types of bacteria present in a water supply depend on the form of contamination. Pure water contains mainly harmless, pigment-producing (chromogenic) bacteria, but typical soil bacteria, such as the bacilli, *Proteus* and *Pseudomonas* may occur, especially from field drainage on arable land. If these latter types are present in large numbers in the water, they may produce faults in milk and its products. In water supplies polluted with sewage, the principal types of bacteria are of intestinal origin and are putrefactive in nature. These forms are particularly troublesome in dairies and creameries in causing faults such as gassiness in milk products. Moreover, in sewage-polluted waters, disease-producing (pathogenic) bacteria may occur in which case the water supply becomes dangerous to health. This is especially so with those diseases where the causal bacteria multiply in the intestinal tract and are, therefore, teeming in the faeces. Such diseases include cholera, dysentery and typhoid and paratyphoid fevers. Outbreaks of these diseases have in the past not infrequently been traced to sewage-polluted water supplies. If such a supply is used to wash cows and to clean utensils, the milk may become contaminated with the pathogenic bacteria and so act as a further means of spreading the disease. This is why a Medical Officer of Health can demand a list of customers from a milk retailer. Water supplies polluted with

these pathogenic bacteria are only safe for direct use after they have been thoroughly boiled or chlorinated.

The best water supply for a dairy or creamery is from the municipal mains, where the bacterial cleanliness is assured by regular routine testing. Failing this, natural spring water or deep well water should be sought, but should be used only after its purity has been confirmed by tests.

TESTING OF WATER SUPPLIES

In determining the suitability of a water supply for dairy use, the types of bacteria present in it are more important than their numbers. It is therefore necessary to combine the results of several different tests before a reliable picture can be drawn up showing the purity of the water. The sample drawn for testing must be fully representative of the supply. Sterile glass sampling bottles with glass or rubber stoppers are used. If the sample is being taken from a tap, the water should be allowed to run for about 10 minutes and then the mouth of the tap scorched with a blow-lamp. After a further few minutes of flowing, the sample may be taken. This procedure ensures that all the bacteria found were originally in the water and did not come from the surfaces of the tap. With spring or river water the sample bottle should be opened under the surface with the mouth of the bottle facing upstream to avoid contamination from the hand of the person taking the sample. Deep well water is sampled by using a weighted bottle with lengths of cord attached both to the bottle and the stopper. The bottle is lowered below the surface and the stopper removed by pulling the cord. Samples should be tested as soon as possible before the bacteria in them start to multiply. If testing must be delayed, the samples should be put in cold store. The following are the principal tests employed in examining a water supply for dairy or creamery use :—

(*a*) *The Plate Count* determines the actual numbers of bacteria in the water. It is performed similarly to a plate count for milk and 1 ml. or suitable dilutions of the sample are " plated out " on an agar medium in Petri dishes. Two plates are incubated at 37 C and two at 22 C. After a suitable incubation period, the bacterial colonies which have developed are counted. The number of bacteria per ml. is calculated by the use of the same methods as for a milk plate count. Counts at 37°C and 22°C are thus obtained for the sample and much can be deduced by comparing them. Unpolluted water has a much higher count at 22°C than at 37°C because most natural water and soil bacteria grow best at the lower temperature. In this case, the ratio of the count at 22°C to the one at 37°C will be at least 10 to 1. On the other hand, sewage-polluted water which contains large numbers of intestinal bacteria suited to growth at 37°C (body heat), will give a ratio of much less than 10 to 1. A water supply fit for dairy farms and creameries should not contain more than about 100 bacteria per ml. when incubated at 22°C. If there are well over 1,000 per ml., the supply should be treated as of doubtful quality until further tests show that only normal water bacteria are present.

(*b*) *The Presumptive Coliform Test* is carried out exactly as for a milk sample except that larger volumes of water are used in each tube. With water, 1, 10, 20, and even 50 ml. portions may be tested whereas with milk, 1 ml. is normally the largest volume used in one tube. The technique of the test is fully described in a later section. As coliform bacteria are intestinal in nature, their presence in a water supply indicates sewage contamination, but gives no reliable indication as to whether the contamination was recent. This is because the *Aerobacter* types of coliform bacteria can remain alive for long periods in soil and water. Water supplies sufficiently pure for dairy use should not contain coliform in less than 10 ml. Water which has been contaminated with sewage contains coliform organisms in varying numbers. In such cases, they are often present in 1/10th ml. and even 1/100th ml.

If the water is found to contain appreciable numbers of coliform bacteria, further tests are generally carried out to determine if the organisms are of the " faecal " or *Escherichia* type or the " non-faecal " or *Aerobacter* type. Tests for this purpose include the Koser Citrate, Methyl Red and Voges-Proskauer tests. As the faecal or *Escherichia* types cannot live for long away from the animal body, their presence in a water supply indicates fairly recent sewage contamination. Non-faecal or *Aerobacter* types may be derived from surface drainage of arable land as well as from remote sewage sources so that less importance is attached to their presence in water than to that of the true faecal types. Nevertheless, if present in large numbers, *Aerobacter* species can bring about the development of taints and other defects in milk and its products.

(*c*) *The Faecal Streptococci Test* detects the presence of *Streptococcus faecalis* in water. As this organism is a commensal of the intestine, it dies very rapidly in water due to the lower temperature and so its detection indicates very recent faecal contamination. Polluted water frequently gives a negative reaction because the bacteria are dead due to their having been away from the animal body too long. Thus no reliance can be placed on a negative result. If, however, *Streptococcus faecalis* are found to be present and alive in the water, this is a sure indication of very recent faecal contamination. It also indicates the possibility of the presence of living pathogenic bacteria and therein lies the test's importance. The technique of the test is described in a later section.

(*d*) *The " Stormy Clot " Test* is used to detect the presence of *Clostridia* in milk or water. These bacteria are normal inhabitants of the animal intestine and of faeces and so indicate sewage contamination, but, as their spores are very resistant, this contamination may have been very remote. The test is carried out by mixing equal quantities of the water under examination and sterile milk. This mixture is put into a sterile flask and a layer of sterile liquid paraffin poured on top to exclude air. The mixture is then pasteurised in the flask by subjecting it to a temperature of 80°C in a water bath for 20 minutes after which the flask is incubated at 37°C. The heat-treatment kills off all the non-sporing bacteria but not the spore-formers. The exclusion of air by the oil layer encourages the growth of the anaerobic

clostridia. If the water contains any clostridia, these will commence to grow and multiply and will coagulate the milk. Later this curd will be split and broken due to considerable gas production by the clostridia. Such a reaction in milk is known as a "stormy clot." The presence of clostridia may be confirmed by microscopic examination of the curd for rod-shaped organisms containing spores which are larger than the mother cells so that the latter are swollen either in the middle or at one end.

The Interpretation of the Results of Tests on a Water Supply.

In all cases, a very high number of bacteria in the water supply should be considered suspicious and if in addition, coliform bacteria are present in less than 10 ml., the supply should not be used in connection with milk production or processing. An idea as to how recent the contamination was can be gained from the results of the other confirmatory tests, especially that for faecal streptococci. In addition to these bacteriological tests, the purity of a water supply may also be deduced to some extent by chemical analysis. This includes estimations of the amounts of nitrates and ammonia in the water, and also the amount of soluble organic matter together with the ratio of the percentage of dissolved oxygen to that of organic matter.

While it is not practicable for individual dairy farms to purify and sterilise their water supplies, large creameries frequently do so. Some of them employ sterilising plant using ozone, which has the advantage of adding no flavour to the water and yet imparting to it a distinct clarity and sparkle.

BACTERIAL TAINTS IN MILK.

In investigating a bacterial taint of dairy products, it is of the utmost necessity to trace the source of the bacteria responsible. The taint itself may be temporarily removed by the meticulous washing and sterilising of utensils and equipment, but until the actual source of contamination itself is removed or nullified, there can be no hope of permanent freedom from the taint. This applies to all bacterial faults of dairy produce.

There are various types of bacterial taints which may occur in milk, and each is caused by a different group of micro-organisms. The following are the commonest bacterial milk taints:—

(1) A *bitter taste* may be caused by certain yeasts growing in the milk or it may be due to casein-digesting bacteria such as *Streptococcus liquefaciens*, which is also responsible for sweet-curdling of milk. Several of the bacilli are also capable of producing bitter flavours due to casein-digestion. If these bacteria are causing the trouble, the bitterness will usually develop at low temperatures, as for instance during cold storage, when the lactic streptococci will have been inactivated and will not, therefore, be competing in the milk with the casein-digesting bacteria. If the fault is due to those bacilli which can form spores, it is liable to occur in pasteurised milk as these spores are not killed by heat-treatment. This is one of the reasons why

pasteurised milk in cold storage tends to go bitter instead of turning sour.

(2) *Burnt or caramel flavours* often render market milk unsaleable and are usually associated with warm weather. This taint is caused by an organism, closely related to the lactic streptococci and known as *Streptococcus lactis maltigenes*. This coccus has occasionally been known to infect the udder, in which case the infected cow should be isolated and milked last. Her milk should not be sold liquid, but, if not fed to livestock, may be used for butter or cheese-making. A burnt taste in the milk frequently disappears if great care is taken to sterilise all the utensils thoroughly.

(3) "*Animal*" *or cowshed flavour* may arise through the milk being heavily contaminated with coliform bacteria.

(4) A "*turnipy*" *flavour* in the milk is not necessarily a food taint brought on by the cow eating frosted roots. It may also be caused by the milk becoming contaminated with various moulds and especially certain soil bacteria of the *Pseudomonas* type. When these grow in milk, they produce small amounts of a volatile substance with a "turnipy" taste.

(5) A *soapy flavour* can persist in milk if soap powders have been used to wash the utensils and then not properly rinsed away. Soapiness in milk has also been known to arise through the action of certain bacteria which can produce ammonia from the milk proteins. This ammonia disperses throughout the milk and gives a soapy sensation to the palate.

(6) A *putrid flavour* is a specially objectionable taint occasionally encountered and caused by the action of certain bacteria which have the power of attacking sulphur and converting it to hydrogen sulphide. This is the substance responsible for the offensive odour of bad eggs. The usual way in which milk can become contaminated with sulphur is by the use of unvulcanised rubber milk tubes on milking machine units.

(7) A *fishy flavour* can arise in milk contaminated with a micro-organism called *Proteus ichthyosmius*. This organism, when actively growing in milk, can produce trimethylamine with its distinctly fishy flavour. This fault is, however, more frequently met with in milk products, such as cream and evaporated milk.

Bacterial milk taints differ from non-bacterial in the following ways :—

(1) Milk with a bacterial taint very often contains large numbers of bacteria and a plate count of such tainted milk may show up to one million bacteria per millilitre if the contamination is very heavy. Milk with a non-bacterial taint never contains such large numbers.

(2) If the taint is present in the milk when it is first drawn, it is most likely to be a food taint and will frequently disappear in part when the milk is left exposed to the atmosphere, or when it is aerated by passing over the cooler. If the taint gradually develops after the milk is drawn, it usually indicates bacterial contamination, but develop-

ing taints also arise through the absorption of volatile substances by the butter-fat in the milk, such as for instance, paraffin or creosote. Milk exposed to direct sunlight may also exhibit a slowly developing tallowy taint, due to oxidation of the fat catalysed by dissolved copper in the milk.

(3) If the taint is bacterial in origin, it can be transferred to sterile milk, as the causal bacteria will continue to grow in the sterile milk and so cause the taint to develop again. A non-bacterial taint cannot be transferred to sterile milk, unless of course, sufficient of the tainted milk is added to influence the flavour directly. Even if this is done the taint does not develop further on standing.

The source of a bacterial milk taint may frequently be traced by conducting a keeping-quality test on the milk of each cow, and testing all utensils, coolers, and milking-machine units, etc. If it is found that on keeping, the milk of any one cow develops a taint, then that cow is the offender and is contaminating the whole milk supply. Similarly, the utensils, coolers and units may be tested by rinsing with sterile water. This water is then inoculated into sterile milk and portions of the latter incubated at 22 C and at 37 C. If utensils are the source of contamination, then sterile milk inoculated with rinsings from them will develop the taint. Milking overalls and stools may also be tested for taint-producing bacteria in this way. Finally and most important of all, the water supply to the cowshed and dairy should also be tested by inoculating a few millilitres of it into sterile milk and noting if the taint develops.

In safeguarding the milk supply against bacterial taints, the following measures should be taken :—

(1) The milk should be produced cleanly to prevent as far as possible, contamination with micro-organisms.

(2) Milk should be cooled below 50 F immediately after milking and held at a low temperature, although cold storage alone will not preserve milk indefinitely.

(3) Pasteurisation is desirable to kill off most of the tainting bacteria and this will also drive off any volatile absorbed taints. But heat-treatment will not kill the spores of bacilli and other sporing micro-organisms, which may then germinate and grow, producing bitter flavours.

(4) If the milk is to be used for butter or cheese, it should be ripened with a vigorous starter at a constant warm temperature. This ripening, by bringing about active lactic acid fermentation, will tend to overcome and kill the tainting bacteria, as they are usually very sensitive to the presence of lactic acid in milk.

If a bacterial taint is giving trouble on a dairy farm, the source of contamination should immediately be traced and nullified otherwise the fault will continually recurr. Extra steaming of utensils and extra washing of the cows with a stronger detergent solution are only temporary measures and merely postpone the tracing of the fault.

Normal Souring

If normal fresh milk is held at a fairly warm temperature such as 60 to 70° F, it will rapidly turn sour. This souring is a natural process and is a form of preservation of some of the milk constituents. As such, it was put to practical use in very early times, for the purpose of preserving milk products such as cheese and the fermented milk beverages, from the actions of undesirable putrefactive bacteria.

Souring is the breaking up of the lactose by bacteria and is commonly called "lactic acid fermentation." The bacteria responsible for souring are collectively known as the lactic acid bacteria. The chief of these are the lactic streptococci and the lactobacilli although certain coliform bacteria and several of the micrococci have lactic fermentative powers. These bacteria attack the lactose and convert it chiefly into lactic acid although small amounts of other acids, such as acetic, may be produced. Coliform bacteria and certain micrococci can also produce gas from the lactose. The lactic acid produced has the effect of coagulating the casein and causing "curdling" which is thus an integral part of the souring process.

At about 1% of lactic acid, the lactic streptococci start to die off, although the lactobacilli will continue to grow and may produce up to about 4% of lactic acid in the milk. If this acidity is neutralised by alkalies such as lime, the bacteria may again become active and continue to ferment the lactose.

The souring bacteria grow best at around 70°F and are a serious cause of loss in uncooled market milk and even in milk which has been cooled but which has stood in churns exposed to the sun, especially in summer. The principles of clean milk production are directed towards excluding these souring bacteria from the milk and checking the growth of any which have gained access. The sources of souring bacteria in raw milk include the air and dust of the cow-shed, the cows' skin, the hands and clothes of the milkers, fodder and bedding and, of course, dirty utensils. All the souring bacteria are greatly checked by cooling the milk.

By producing lactic acid, these bacteria overcome and inhibit putrefactive and disease-producing bacteria in the milk and tend to preserve the fat and protein. This preservation is the basis of cheese-making and also plays an important part in those methods of butter-making where the cream is ripened prior to churning. Souring is also made use of in the production of fermented milk drinks such as Kefir, Koumiss and Yoghourt.

Abnormal Milk Fermentations.

In addition to natural souring, there are several other types of fermentation which may occur in market milk, especially when it is heavily contaminated with soil or intestinal bacteria. In such cases, the lactic acid bacteria cannot overgrow the others and so-called

" abnormal milk fermentations " arise. The commonest of these are as follows :—

Gassy fermentation is brought about by the milk becoming heavily contaminated with yeasts or coliform bacteria. These organisms can ferment the lactose in the milk with the production of volumes of gas as well as lactic acid. This gas is mainly carbon dioxide with a little hydrogen. The production of gas in the milk leads to the formation of bubbles. Fermentation such as this is one of the principal causes of churns of milk, and in pre-war days, of cream, " foaming-over " in transit. " Foaming-over " is worst where the churns are being transported long distances in warm weather. This is especially so where coliform bacteria are the cause. These organisms are intestinal in origin and so grow best in milk which has not been cooled or which has been warmed again by the sun. Gassy fermentation is also troublesome in butter-making where it causes " sleepy " cream, which does not churn well, and in cheese-making, where it produces floating or " pin-hole " curd. Bacteria responsible for gassiness in milk gain access from dung, silage, fodder and bedding and through the utensils, cows' coat and the milkers' hands.

The true yeasts or *Saccharomyces* do not ferment lactose and therefore do not cause gassiness in milk and cream. The *Torula* yeasts do; they enter the milk from the cow's skin, dust in the air and badly-sterilised utensils. These organisms produce lactic acid, alcohol and much carbon dioxide as well as yeasty flavours. Like coliform bacteria, they grow best at blood heat and are therefore, most active in warm milk.

Gassiness in milk and cream may be controlled by attention to the following recommendations :—

(1) The milk should be produced as cleanly as possible with a view to excluding yeasts and coliform bacteria. All utensils should be thoroughly sterilised, preferably by steam, at least once daily.

(2) The milk should be cooled and stored below 50 F as the " gassy " organisms do not grow at such low temperatures.

(3) If the milk has to be transported over long distances it should be pasteurised to destroy any bacteria and yeasts in it. Similarly if the milk has to be kept and used for butter-making, the cream should be pasteurised.

(4) If the milk is to be made into cheese, it should be ripened with a pure and vigorous starter at a relatively low temperature. This will encourage the lactic streptococci to overcome any yeasts and coliform bacteria. In cheese-making, gassiness is always worst where the initial ripening has been slow so that insufficient acid has been produced. If a starter becomes gassy, this indicates contamination with coliform bacteria or yeasts. Such a starter should be discarded at once.

Butyric fermentation is caused by sporing anaerobic bacteria of the genus, *Clostridia*. These micro-organisms can attack lactose with the production of butyric acid. This acid imparts to the milk

a characteristic and extremely offensive odour. Milk which has undergone butyric fermentation thus has a foul putrid taste and smell, and is completely useless for the liquid market. Moreover, as the flavour is absorbed by the butter-fat, the milk should not be made into butter.

The causal bacteria occur in the soil, in the animal intestine and in dung and also in sewage-polluted waters. They gain access to the milk from these sources, through the use of improperly sterilised utensils and from the cow's coat. As they are anaerobic, they will not grow if air is dissolved in the milk and they are thus usually only active when milk is rapidly going sour. In this case the lactic acid bacteria are quickly using up the dissolved oxygen so permitting the growth of the clostridia. These clostridia are acid-tolerant and therefore will not be inhibited by the acid in sour milk. If liquid milk is delayed in transit for long periods in warm weather, it may turn sour normally and then undergo butyric fermentation.

Pasteurisation will not kill the spores of the clostridia and as the air is driven out of the milk during the heat-treatment, the spores frequently germinate after pasteurisation and bring about butyric acid fermentation, especially if the milk is stored in a warm place. Under certain conditions pasteurisation may actually assist butyric fermentation by removing all controlling competition from non-sporing bacteria.

Prevention of butyric fermentation in liquid milk consists mainly in the production of clean milk with special attention to the avoidance of contamination with dung and sewage-polluted water. Although pasteurisation does not kill the spores, it often does assist by killing off all the vegetative forms of the clostridia. As the bacteria are intestinal in nature they grow best at blood heat. Cold storage of the milk after heat treatment will prevent the development of any surviving spores.

Butter and other milk products containing fat may become tainted with butyric acid produced by aerobic fat-splitting bacteria and fungi. Rancidity in butter may be caused in this way. Such a butyric taint should not be confused with the true "butyric fermentation" of lactose brought about by clostridia. The presence of clostridia in milk may be detected by the "Stormy Clot" test. This involves pasteurisation of the milk followed by incubation under anaerobic conditions to encourage the growth of any spores of clostridia. The technique of the test is described in another section.

Acetic fermentation may occur in liquid milk either through coliform bacteria, or due to the growth of lactic acid organisms under unfavourable conditions. Acetic acid may be produced either from the lactose or from lactic acid, and its presence imparts a sharp, vinegary flavour to the milk. This abnormal fermentation usually occurs in old sour milk where the developed acidity is high enough to be unfavourable for the growth of lactic streptococci. It also occurs frequently after an outbreak of gassiness caused by heavy coliform contamination. Control measures to prevent the development of acetic acid in milk are similar to those for gassiness, and are centred on the production of clean milk using efficiently sterilised utensils

followed by prompt and thorough cooling. Acetic acid fermentation in starters can easily be prevented by propagating the starter cultures daily, to avoid excessive acidity unfavourable to the lactic streptococci.

These three abnormal fermentations can cause much spoilage and loss of liquid milk especially in warm weather and when milk is being transported long distances. In the manufacture of certain Swiss cheeses, however, some of these fermentations are encouraged and the starter cultures actually contain the specific type of bacteria required.

ROPY MILK

This is one of the most persistent and insidious changes which can take place in liquid milk. It may occur in sweet or sour milk, in butter-milk, starters or even in whey. The fault is known as " ropiness," " sliminess " or " stringiness " and it may be so slight as to be almost imperceptible. Usually, however, " ropy " milk is easily detected when it is poured from one contianer to another. In bad cases the milk can be drawn out into long, stringy threads when a stick is dipped into it. Occasionally, milk may become thick and doughy. The fault is entirely bacterial and many different types of bacteria may be responsible. It should not be confused with a similar condition in milk from a cow suffering from mastitis. This latter milk is often slimy and stringy and is commonly known as " thick milk," but its abnormality is due to the large numbers of leucocytes (white blood corpuscles) present, and also to its very high fibrin content.

Usually ropiness develops after milk has been stored for several hours during which the causal bacteria have grown and multiplied. Ropiness or sliminess may occur only after the milk has soured or it may disappear when the milk turns sour, depending on the type of bacteria responsible.

The ropiness may be caused in various ways. If the bacteria exist in the form of long chains, as with certain lactic streptococci and lacto-bacilli, and are present in very large numbers the milk will appear stringy. If the bacteria in the milk cover themselves with gelatinous sheaths or capsules, these will slide over one another in the milk and give a sensation of sliminess. If the bacteria are in long chains as well as forming capsules, the milk will be both slimy and stringy. Lastly, certain putrefactive bacteria by breaking down the casein produce mucins and gums, which impart a sliminess to the milk.

If the casual bacteria are aerobic, ropiness will develop only on the surface of the milk in the containers. Indeed the fault is frequently confined only to the cream layer for this very reason. Similarly, if the bacteria responsible for a particular outbreak of ropiness grow best at low temperatures—and most of them do—the fault will only develop when the milk is stored in a cold place, that is below 50° F. Above 70°F, the lactic acid bacteria will outgrow and overcome the " ropy " bacteria thus preventing the development of the fault.

Ropiness or sliminess may develop so slowly that the milk may not be noticeably affected until after it has reached the consumer

and the producer may be completely unaware of the presence of the fault. Commercial starters may become slimy at low temperatures due to the poor growth conditions for the lactic streptococci. If this happens the starter should be discarded at once.

Bacteria which can cause ropiness in milk include lactic streptococci, lactobacilli and micrococci, but the most common causes are the *Aerobacter* type of coliform bacteria and *Alcaligenes viscosus*. These bacteria frequently occur in milk in small numbers, but they are normally rapidly overcome by the true lactic streptococci, so that the fault occurs only if the number of ropy contaminants is very high or if the milk is stored under conditions unsuitable for lactic streptococci but favourable to the " ropy " bacteria. Such conditions occur when the milk is stored at fairly low temperatures.

If an outbreak of ropy milk occurs on a dairy farm, the source of contamination must be traced at once and removed or nullified, otherwise the fault will persist. It is often possible to check the ropiness temporarily, by very thorough and meticulous washing and sterilising of the utensils, but unless the true source of contamination is investigated and removed, the ropiness will eventually return, often with increased severity.

The bacteria capable of causing ropiness in milk are not pathogenic (disease-producing) and they are very rarely present in the udder and, therefore, in the milk when it is drawn. Isolated and exceptional instances are, however, on record where micro-organisms capable of causing ropiness have been found as commensals in the udder.

The commonest source of contamination with " ropy " bacteria is a dirty water supply and this is always the first suspect when an outbreak of ropiness in milk is investigated. Leaking coolers in the farm dairy are also a common source of ropiness but only if the water supply is contaminated. Leaks in a cooler may be readily detected by filling it with water when the leaks will be apparent as very fine jets or sprays. If the dairy herd is ranging over a flooded pasture, the cows frequently pick up the bacteria on their udders and the micro-organisms later gain access to the milk and bring about ropiness. There is an old belief that ropiness is linked with certain weeds in pasture, such as butterwort, but these are invariably weeds of marshy ground and the ropiness is in all probability not due to the weeds themselves but to the wet nature of the land upon which they are growing. Wet, water-logged land often contains large numbers of " ropy " bacteria.

If the causal bacteria form capsules, they " seed " on to the metal surfaces of the utensils and are extremely difficult to remove. Moreover, they are protected by their capsules and normal sterilisation does not kill them so that they can repeatedly contaminate the milk with which they make contact.

In order to arrest and eliminate an outbreak of ropiness, attention should be given to the following recommendations :—

(1) The water supply to the dairy and cowshed and also to the field troughs should be tested for contamination. If it is contaminated,

the source of the bacteria should be traced and nullified. This can be done by chlorination of the reservoir tanks and all water-pipes with a solution of sodium hypochlorite at a strength of 1 oz. of stock hypochlorite detergent per 10 gallons of water. Frequently, however, on a farm, it is very difficult indeed to eliminate such natural contamination or to chlorinate the supply . In this case the supply should not be used in the cowshed or dairy and the troughs in the fields should be shut off. This is a very drastic step to have to take but it is of the utmost necessity if the ropiness is to be averted and the water cannot be freed of contamination.

(2) The flanks and udders of the cows should be carefully washed with a detergent solution before milking to kill any " ropy " bacteria clinging to these parts.

(3) The cowshed should be kept clean. All stools, clothes and brushes liable to be contaminated from the water or soil should be scrubbed and disinfected. The hands of the milking personnel should be kept clean by scrubbing with disinfectant soap.

(4) Cooling the milk is not always effective as most of the causal bacteria grow best at low temperatures. If however lactic bacteria are causing the ropiness, cooling will be effective.

(5) Pasteurisation will kill off the " ropy " bacteria as they do not form spores, but milk must be pasteurised as soon as possible and before the ropiness or sliminess appears. If ropiness occurs in pasteurised milk, it indicates either that the heat-treatment has been faulty or that post-pasteurisation contamination has occurred. In the latter case the bottling machine is very often to blame as it is difficult to sterilise properly.

(6) If the milk is to be used for the manufacture of cheese or butter, ripening should be carried out quickly with a vigorous starter. The resulting rapid production of lactic acid will inhibit most of the " ropy " bacteria and so prevent the fault from developing. Acidity, however, will not prevent the growth of those types of lactic streptococci or coliform bacteria causing ropiness.

SWEET-CURDLING AND CASEIN-DIGESTION

This is a bacterial fault in milk which causes curdling without the production of lactic acid. This " sweet " curd is often later digested to form soluble nitrogenous compounds which have bitter flavours. Thus the curd is liquefied and disappears.

All the bacteria which can cause this fault secrete two enzymes. The first is rennin-like in nature and coagulates the casein while the second is proteolytic and digests the coagulated casein into soluble substances. The combined action of the two enzymes is very similar to the action of the rennin and pepsin in the stomach of the milk-fed animal. In some cases the digestion of the casein follows so rapidly after the coagulation that the actual " sweet-curdling " may not be detected and the first symptom of the fault may simply be the development of bitter flavours.

As with ropiness, most of the bacteria causing sweet-curdling and casein-digestion frequently occur in milk in small numbers, but do not cause the fault to develop as they are very sensitive to the presence of lactic acid. At low temperatures, when the lactic acid bacteria are growing only feebly, the sweet-curdling bacteria may gain the upper hand and produce the typical symptoms of the fault. Many of the causal bacteria form spores which can easily resist pasteurisation, so that sweet-curdling and casein-digestion may occur in old pasteurised milk.

The initial symptoms of the sweet-curdling are very similar to normal souring, but the two may be easily distinguished by determining the acidity of the milk at the time of coagulation. In order for normal acid curdling to take place, the milk must contain between 0.5 and 0.7% of lactic acid. Sweet curdling often occurs in milk of perfectly normal acidity. There is one particular sweet-curdling micro-organism, *Streptococcus liquefaciens*, which produces lactic acid in the milk *after* the sweet-curdling has taken place. This secondary acid production may convey the impression of curdling due to normal souring.

The principal bacteria which cause sweet-curdling and casein-digestion are bacilli. These occur in dust, soil, bedding and hay and gain access to the milk via the dust in the atmosphere and on the cow's coat and through the use of unsterile utensils. They are very sensitive to acidity and are quickly inhibited in milk by the lactic acid bacteria but, as they form resistant spores, they can survive heat-treatment and later produce sweet-curdling in pasteurised milk. Milk contaminated with these bacteria is bitter in flavour, gives a soft, bad-flavoured cheese and does not churn easily into butter.

Other bacteria causing sweeting-curdling are *Proteus vulgaris*, *Pseudomonas fluorescens* and as mentioned above, *Streptococcus liquefaciens*. None of these types form spores so they are all killed by pasteurisation. Except for *Streptococcus liquefaciens* they are easily overcome and inhibited by the lactic acid bacteria.

Sweet-curdling and casein-digestion can completely spoil batches of milk for the liquid market. Control measures to prevent the fault are as follows :—

(1) The production of clean milk with special reference to the prevention of contamination from dust from bedding and fodder and also from the coat of the cow.

(2) The use of a pure water supply. Some sweet-curdling bacteria are natural inhabitants of the soil and they may be found in water supplies containing land drainage. Such water can easily contaminate milk through the cooler and other utensils. In the case of an outbreak of sweet-curdling on a dairy farm, the water supply should be among the first of the possible sources of contamination to be tested. If it is contaminated and cannot be conveniently chlorinated to kill the bacteria, it should not be used in connection with milk production.

(3) If the milk is to be made into butter or cheese, ripening should be carried out with a vigorous starter and at a fairly high

temperature such as 75°F. This high temperature of ripening together with the active production of lactic acid will kill off or seriously inhibit all the sweet-curdling bacteria with the exception of *Streptococcus liquefaciens.*

DISEASES CARRIED BY MILK

It is impossible to produce milk which does not contain bacteria. If the cow is diseased, bacteria of that disease may be present in her milk. Common instances of this are tuberculosis and contagious abortion; the bacteria of the latter cause undulant fever in humans. Diseased personnel handling milk and coughing over coolers and other equipment can also introduce pathogenic bacteria into milk. Diseases such as scarlet fever and dysentery frequently gain access to milk in this manner. Moreover, cows and persons suffering from a disease in a sub-clinical condition, that is, where there are no obvious symptoms, may contaminate milk with these particular disease bacteria. Carriers of a disease, that is, where the cow's or person's body harbours the disease-producing bacteria and yet does not actually suffer from that disease, are also capable of contaminating milk. These carriers are particularly dangerous as they are very difficult to detect and they may go on contaminating the milk for months before they are discovered. There are many cases on record where large outbreaks of milk-borne disease have been traced to one single person who was a carrier of that disease and who was handling the milk supply and at the same time repeatedly contaminating it.

Raw milk and even pasteurised milk can never be completely safe, as carriers of disease and also sub-clinical cases are entirely outside the producer's or dairyman's control. If, however, milk production and distribution are carried out under hygienic conditions with healthy cows and personnel all of whom are regularly tested for health, then the danger of pathogenic bacteria being in the milk is greatly lessened although not altogether eliminated.

Characteristics of Outbreaks of Milk-borne Diseases.

(1) These are frequently epidemic in nature. Cases "blossom" out simultaneously and are often confined to households receiving the same milk supply. This is not always the case however, as the producer of the milk contaminated with the pathogenic bacteria, may supply several dairies which may or may not pasteurise the milk they receive.

(2) Outbreaks of these diseases often "explode" suddenly. Large numbers of cases develop together and then die away when the source of contamination is discovered and corrected. If not, they continue in a steady stream. This also applies to an outbreak of disease due to a polluted water-supply or to infected milk products, especially ice-cream.

(3) If the disease is milk-borne, the proportion of infected persons will tend to be greater in households using large quantities of milk than in houses using very little milk, especially if there are children in the house. This does not always hold true as certain households

may boil their milk before consumption and other families may have a natural resistance to the disease.

(4) Outbreaks of milk-borne diseases are commoner in villages than in towns as pasteurisation is not so widely carried out and there is a less rigid control of milk handling and distribution.

In tracing the source of an outbreak of a milk-borne disease, there is little to be gained from testing the actual milk supply itself as the pathogenic bacteria may only occur spasmodically in the milk and in any case, they are usually present only in very small numbers. Moreover, the laboratory procedure necessary to detect, isolate and identify any pathogenic bacteria takes several days and this gives the outbreak time to spread still further.

The usual method of tracing the initial source of contamination in a suspected milk supply is first to study details of the outbreak with reference to the distribution of cases in households, etc. and then one by one to eliminate the possible sources of contamination. Although milk and its products are always the first suspect, polluted water supplies, raw vegetables and foodstuffs exposed to flies may also be responsible for the disease outbreak. If the milk supply has proved to have been at fault, the main source of contamination may be :—

(1) The cow herself, as she may be suffering from the disease or may be a carrier.

(2) The milking personnel may likewise be diseased or be acting as carriers.

(3) The water-supply may be polluted with sewage or faeces and so contain the bacteria responsible for the disease.

(4) Contamination may be coming from a customer's household by way of contaminated milk bottles.

The relative importance of the various possible sources depends on the nature of the disease itself and whether the bacteria of the disease occur in the faeces or in the saliva, and whether these bacteria can resist heat, acid and perhaps low temperatures.

Prevention of the Spread of Pathogenic Bacteria in Milk.

While it is impossible to produce milk guaranteed to be free from all disease-producing bacteria, there are various processes, which if properly carried out, will reduce the risk of the milk being rendered dangerous by pathogenic bacteria. These are :—

(1) Cooling the milk. This will not kill bacteria, but if the milk is efficiently and quickly cooled, the growth and multiplication of the pathogenic bacteria is drastically checked. All milk should be cooled to at least 50°F and preferably lower.

(2) Pasteurisation will kill all the non-sporing disease-producing bacteria. Sporing types are not usually present in milk. It is a good, cheap safety measure but must be performed efficiently, as it destroys all competition by the normal lactic acid bacteria against any disease-producing bacteria gaining access to the milk after the heat-treatment. This later contamination is, however, fairly easy to avoid as all handling

of pasteurised milk is mainly mechanical so that few persons have an opportunity to contaminate the milk.

If these two operations are carried out quickly and thoroughly, the milk will be as safe as is possible without actual sterilisation. In general, the production of milk free from disease-producing bacteria may be summarised as follows :—

(1) Cleanliness in the cowshed and dairy. The utensils must be kept sterile and the atmosphere of the buildings should be pure and sweet. All surfaces in the cowshed should be kept clean and free from contamination with dung. Dung is a very likely source of those bacteria which cause intestinal diseases.

(2) Both the cows and the milking personnel should be healthy and should be tested periodically to detect carriers and sub-clinical cases. Special watch should be kept for persons handling milk who are suffering from coughs.

(3) The water-supply must be free from pathogenic bacteria and should be tested for such at intervals. The water should also be tested for traces of sewage-contamination. Many outbreaks of typhoid fever and cholera have in the past been traced to milk which has become contaminated with sewage-polluted water.

(4) The milk should be cooled to at least 50° F as soon as possible after production in order to inhibit the growth of bacteria, including pathogenic types.

(5) Pasteurisation should be carried out and the milk stored at a low temperature afterwards.

All these precautions will, of course, prove completely useless if the consumer empties her bottles of milk into dirty, unhygienic jugs, which may possibly be contaminated with pathogenic bacteria. Human diseases whose outbreaks are frequently milk-borne are as follows :—

Cholera	Tuberculosis
Dysentery	Diphtheria
Typhoid fever	Scarlet fever
Paratyphoid fever	Septic sore throat
Undulant fever	Infantile summer diarrhoea
Gastro-enteritis	

and also possibly, measles and infantile paralysis. These disease bacteria have been known to occur in raw milk but very seldom in supplies of pasteurised milk.

SECTION II.—PROCESSED MILK AND MILK PRODUCTS.

PASTEURISATION

This is a heat-treatment carried out on milk with a view to destroying all the pathogenic and souring bacteria, and thus rendering the milk safe and of improved keeping-quality. The heat-treatment must not be so severe as to alter the flavour and cream-line of the milk. Cooling after heating is an essential part of the process as all the bacteria in the milk are not killed by the heating, and any survivors will continue to grow vigorously in the milk if it is left warm.

As the rate of destruction of the bacteria increases with rise in temperature during the heat-treatment, the efficiency of pasteurisation depends on the temperature reached and the time the milk is maintained at this temperature. Various combinations of time and temperature may be used to pasteurise milk but only two are officially recognised for the Graded milks. These are (*a*) the *Holder Process* in which milk is maintained between 140° and 145°F for at least 30 minutes and (*b*) the more modern *High-Temperature Short-Time Process* (H.T.S.T.) in which milk is heated to at least 162°F for at least 15 seconds. In both cases the milk must be cooled immediately after heating, to 50°F in Scotland, and 55°F in England and Wales. Most large pasteurising dairies and creameries cool their milk down to about 40°F by mechanical refrigeration. In the case of High-Temperature Short-Time pasteurisation, the plant must be equipped with thermostatic control and must have an automatic flow diversion valve to detect and divert any milk which has not been properly treated. One of the first methods of heat-treatment was the so-called " Flash " pasteurisation where the milk was simply heated up to about 165°F and then cooled. This method has never been officially recognised as exact heat control is very difficult and the milk may take on a slightly cooked flavour. " Flash " pasteurisation is still used, however, on milk for cheese-making.

With market milk the effect of pasteurisation on the flavour and cream-line is fully as important as the efficient killing of bacteria. The consumer places more reliance on the appearance of the milk and its taste than on whether the milk contains many bacteria or not. The two officially recognised methods of pasteurisation, if properly carried out, do not affect the flavour or cream-line and yet they kill all the pathogenic bacteria. Efficiency in the destruction of disease-producing organisms is indicated by the destruction of the natural milk enzyme, phosphatase. This enzyme, present in all raw milks, is inactivated at a slightly higher temperature than that required to destroy the tubercle bacillus, which is the most resistant type of non-sporing pathogenic micro-organism to be found in milk. Thus if the phosphatase is destroyed by the heat-treatment, so also must all the non-sporing pathogenic bacteria. The technique of the phosphatase

test is described in another section. Experiments have shown that the tubercle bacillus is destroyed with a good safety margin in both methods of pasteurisation.

To be successful, the heat-treatment must not only kill off the pathogenic bacteria but must also effect a large reduction in the total numbers of bacteria in the milk. Usually between 97 and 99% of the milk bacteria are destroyed, but for any given time and temperature of heating, the higher the original number of bacteria in the milk, the greater will be the percentage reduction. With the Holder process a skin may form on the surface of the milk. Any bacteria in this skin are protected from destruction. If the milk is agitated to prevent this skin forming and froth develops, then any bacteria in the froth will also be protected to some extent.

Although pasteurisation does not kill the spores of sporing bacteria, these are not usually present in milk. Most of the survivors of heat-treatment are therefore non-sporing types and are either *thermophilic*, that is, they can actively grow and multiply at pasteurisation temperatures or they are *thermoduric*, that is, they can easily tolerate pasteurisation temperatures, although they do not grow during the process. Bacteria which can survive pasteurisation may conveniently be classified into three groups according to their action on milk :—

(1) Inert bacteria with little action.
(2) Acid-producing bacteria.
(3) Proteolytic bacteria.

In normal raw milk, the percentage of each group on the average, is 60, 30 and 10 respectively, while in pasteurised milk, the percentages are about 75, 20 and 15. In old pasteurised milk, the proteolytic group tend to develop more than the acid producing group. In raw milk, the acid producing bacteria develop vigorously at the expense of the proteolytic bacteria. This explains why pasteurised milk tends to go " bad " or putrid while raw milk tends to go sour.

Organisms of the first group have little effect on the milk beyond forming small amounts of alkaline substances. They are either thermophilic or thermoduric. Their presence in pasteurised milk gives no indication of faulty pasteurisation or of post-pasteurisation contamination, as they occur equally frequently in both raw and pasteurised milks. The group comprises chiefly micrococci and staphylococci.

The second group contains by far the largest number of types of bacteria as it includes all the true and pseudo-lactic acid bacteria, as well as the butyric acid bacteria. The spores of these latter clostridia are not killed by pasteurisation and may later produce butyric acid fermentation in the pasteurised milk. They may give a positive reaction in the coliform test in pasteurised milk, but may easily be distinguished by the " Stormy Clot " test. True coliform bacteria rarely survive pasteurisation, but certain specially heat-resistant strains may do so. Any heavy coliform contamination of pasteurised milk thus generally indicates faulty heating or contamination after the heating process. This post-pasteurisation contamination may arise in various

ways. There may be a leakage of raw milk into the pasteurised milk, or the coolers, bottling machines or bottles may be dirty. The creamery personnel may also contaminate the milk if their hands are not clean. Lactic streptococci and lactobacilli should not be present in pasteurised milk as they are not heat-resistant. If they are present post-pasteurisation contamination is indicated. Some bacteria closely related to the true lactic streptococci, such as *Streptococcus thermophilus*, and also *Lactobacillus thermophilus*, can survive pasteurisation. Indeed, *Lactobacillus thermophilus* can actively grow and multiply at the temperature of the Holder process. Thermoduric lactic acid bacteria, such as *Streptococcus thermophilus* and *Lactobacillus casei* can resist the temperatures of pasteurisation, but will not grow during the process. These produce acid slowly in pasteurised milk but in raw milk they are usually overgrown by the normal lactic acid bacteria.

Group 3 includes sporing bacilli. The spores of these micro-organisms can easily survive pasteurisation, but they are not usually present in raw milk. If they do occur in large numbers, they will produce defects in the pasteurised milk. Non-sporing survivors of pasteurisation in this group include *Proteus* and *Pseudomonas* types. These may either be the remnants of heavy contamination of the original raw milk or they may have been introduced by post-pasteurisation contamination. The total numbers of survivors of this group in pasteurised milk is always low.

THERMOPHILIC AND THERMODURIC BACTERIA IN PASTEURISED MILK

The above three groups of survivors of pasteurisation contain both thermophilic and thermoduric bacteria.

Most of the thermophilic bacilli are "sweet-curdling" micro-organisms, but a few produce acid in milk. Thermophilic bacteria are most numerous in milk pasteurised by the Holder process. Reductase tests carried out at 145°F only detect those which happen to reduce dyes. The presence of thermophilic bacteria in pasteurised milk is often due to the formation of a skin or froth on the surface of the milk during the 30 minute period of holding at 145°F. It may also be due to the leakage of small quantities of raw milk into batches of pasteurised milk. Moreover, small quantities of pasteurised milk in the "dead-ends" of pipes or left in the holder between batches of milk mean that bacteria in such small volumes of milk are kept at a high temperature for far longer than usual and this encourages thermophilic bacteria. If Holder plants are run for long periods they generally contain large numbers of thermophilic bacteria at the end of the run. Holder plants should therefore be steam sterilised after every six hours of use at least. Incomplete sterilisation of the equipment will lead to very high numbers of thermophilic bacteria in the pasteurised milk.

If large numbers of thermophilic organisms are found, the source of contamination must be traced. Farm supplies of raw milk containing large numbers of such bacteria can be detected by incubating the milk at 55°C and determining the numbers of bacteria which will grow at

that temperature. Thermophilic bacteria in milk are never derived from the cow's udder but usually from badly sterilised utensils and dirty methods of production. If tests show that the number of such bacteria in the milk increases as the milk flows through the pasteurising plant, then the contamination is coming from the plant.

Thermoduric bacteria commonly found in milk include micrococci, streptococci and bacilli in that order of occurence. The micrococci are inert and of little importance in the milk but the streptococci. such as *Streptococcus thermophilus*, produce acid while the bacilli are proteolytic in nature. Thermoduric bacteria are mostly slow producers of reductase, hence the methylene blue and resazurin tests do not readily reveal their presence. The best estimate of the numbers of thermoduric bacteria in milk is obtained from a plate count carried out at 30 C for 4-5 days. The technique of this test is described in a later section. The source of thermoduric bacteria in pasteurised milk may be traced by taking samples of milk from the farm and at various points in the pasteurising plant and applying the above test. On the farm the various likely sources may be swabbed or rinsed with sterile water and the test carried out on the rinsings. On the whole, thermoduric bacteria have little effect on the safety or keeping quality of pasteurised milk, but their presence in large numbers in the milk indicates dirty methods of production. The commonest source of thermoduric bacteria is improperly sterilised utensils, especially dirty milking machines.

Comparison of the Holder and H.T.S.T. Methods of Pasteurisation.

Both methods are satisfactory from the point of view of maintaining the cream-line and the flavour of the milk. The Holder method has, however, a greater margin of safety for the destruction of pathogenic bacteria than has the H.T.S.T. method. The latter therefore must have very strict and delicate control devices. Also the H.T.S.T. method may very slightly lower the food value of the milk by causing partial precipitation of the lactalbumen.

The Holder method provides much more suitable conditions for the growth of thermophilic bacteria than the newer method and the equipment required for it is bulky, difficult to sterilise and requires much labour whereas the H.T.S.T. plant is compact, easier to clean and also provides a continuous flow of milk to the bottling machines.

The Advantages and Disadvantages of Pasteurisation.

Pasteurisation is beneficial as it destroys the disease-producing bacteria and large numbers of the other bacteria in the milk, which is thus rendered safe for consumption and of vastly improved keeping quality. The flavour and composition of the milk are not greatly affected if the heat-treatment has been properly carried out. There may even be a slight increase in the digestibility of some of the milk proteins. If the tubercle bacillus were alone concerned, pasteurisation of Tuberculin Tested milks would be unnecessary, but other pathogenic bacteria occur in milk also such as *Brucella abortus*, and Tuber-

culin Tested milk is just as likely to contain these others as ordinary milk.

Allegations against pasteurisation are, that if the milk is improperly heated and very dirty, the surviving bacteria have no competition to face as the lactic acid organisms will all have been killed. Thus abnormal fermentations may arise. If the heat-treatment is properly carried out and the milk is of good bacteriological quality, these fermentations very seldom occur. It is true that if the milk is originally very dirty, pasteurisation will only kill the bacteria and leave their products in the milk, but with such milk, the position would be far worse if the milk were left raw. Pasteurisation does destroy some of the natural milk enzymes but these are of doubtful value. One great disadvantage of pasteurisation is that it destroys certain of the vitamins in the milk. Twenty per cent of the vitamin C and 15-25% of the vitamin B_1 present may be destroyed, but vitamin C is also destroyed in milk if it is exposed to sunlight. The chemical composition of the milk may deteriorate very slightly with heat-treatment as about 5% of the lactalbumen is precipitated. The soluble calcium and phosphorus in the milk may be decreased by about 5% also, while a very small amount of the iodine may be volatilised. There may also be a very slight decrease in the cream-line. There is, however, no loss of vitamins A and D and no loss of the availability of calcium and phosphorus.

Pasteurisation is, on the whole, a highly desirable safety measure applied to milk and could with advantage be carried out on all milk of all Designations. The only grade of milk which could be justifiably exempted from pasteurisation is Tuberculin Tested milk produced and bottled on the farm.

STARTERS

In cheese-making and some forms of butter-making, lactic acid fermentation or " souring " is encouraged by the addition of " starters " to the milk or cream. Starters are cultures of the most desirable types of lactic acid streptococci grown in sterilised separated milk. Commercial starters in Britain mainly consist of a mixture of *Streptococcus lactis* and *S. cremoris*, while *S. citrovorus* and *S. paracitrovorus* frequently occur as contaminants. For the manufacture of certain Continental cheeses, such as the Swiss Emmenthaler, other types of bacteria are included in starters.

The advantage of using starters in cheese and butter-making lies in the much quicker production of lactic acid, which inhibits the growth of undesirable putrefactive bacteria. If the milk is pasteurised before adding the starter, few undesirable organisms will be present. The use of starters also permits the control of lactic acid production and thus results in improvement of the quality of the cheese.

If starters are kept too long, the lactic streptococci eventually produce sufficient acid to destroy themselves. To avoid this, starters should be propagated daily. This is done by adding a small amount of the previous day's starter to freshly separated milk which has been thoroughly and carefully sterilised. The lactic acid bacteria grow best in starters if they are incubated at 22°C (72°F) or kept in a uniformly warm room at between 65 and 70°F.

Starters contaminated with coliform bacteria will show traces of gas bubbles in the curd, which will be softer and less smooth than normal. Starters should have a clean sharp acid smell and any starter which has an off-flavour should be discarded immediately. The normal acidity of starter when ready for use is between 0.9 and 1% (expressed as lactic acid).

BACTERIA IN CREAM

The types and numbers of bacteria in cream depend mainly on the quality of the original milk, the age of the cream, whether it was mechanically separated and whether it was pasteurised. Cream is liable to contain large numbers of bacteria as the micro-organisms tend to cling to the fat globules during the process of separation. Gravity methods of separating milk, that is, allowing the milk to stand in pans until the cream rises naturally, are not very satisfactory from a bacteriological point of view, because of the long period taken for separation which provides the existing bacteria in the milk with ample scope for growth and multiplication. Mechanical separation gives, by far, the cleanest cream as the milk is usually separated soon after it is produced, so that there is little time for bacteria to grow in it. In a centrifugal separator, many of the bacteria collect in the separator slime together with the dirt and most of the leucocytes. Others stick to the fat globules and collect in the cream. Thus the separated milk usually contains very few bacteria.

Cream contains little lactose so that production of lactic acid is slow, and, although it contains a high percentage of butter-fat, the occurrence of fat-splitting organisms in cream is uncommon. The numbers of bacteria in cream vary widely and in the case of unripened cream obtained by one of the gravity methods, may attain to millions per millilitre. Cream ripened by means of a starter may initially contain a very high number of bacteria due to the large numbers of lactic streptococci in the starter, but after ripening for between 24 and 48 hours, the numbers of lactic acid bacteria begin to decrease. They are gradually killed off by the lactic acid which they have produced. If ripening were continued for about seven days, there would be relatively few bacteria left alive in the cream.

In modern creamery practice, cream for butter-making is not ripened. Pasteurisation, followed by chilling the cream in cold store, gives much better control over the quality of the butter produced, as it destroys the bacteria in the cream. In farm-house and other small-scale butter-making, it is still very desirable to ripen the cream by adding starter to it. The reasons for this are :—

(1) During ripening, the lactic acid bacteria present in the starter produce lactic acid. This kills off undesirable bacteria and so assists in the production of a butter of better keeping quality. Moreover, the active production of lactic acid almost completely inhibits the growth of any putrefactive bacteria present which might otherwise produce taints in the butter.

(2) The presence of lactic acid assists churning by lowering the surface tension of the cream. This facilitates aggregation of the fat globules into butter granules. The flavouring material " diacetyl " is formed during ripening.

The main types of bacteria found in cream are predominantly lactic streptococci, and if the cream has not been pasteurised, coliform bacteria. During ripening, *Streptococcus lactis* and *S. cremoris* (the two " true " lactic acid bacteria) ferment the lactose into lactic acid and later into citric and other organic acids, while *Streptococcus citrovorus* and *S. paracitrovorus* (the " associated " lactic acid bacteria) attack the citric acid which the first two types have produced, and convert it into diacetyl. Most commercial starters contain the above four types of lactic streptococci. Raw, unpasteurised cream ripened without the addition of starter usually contains numbers of putrefactive bacteria as there are not enough lactic acid bacteria to keep them in check. Thus butter made from this type of cream usually has inferior flavour and keeping-quality and is liable to taints. In carefully pasteurised and ripened cream, if the percentage of lactic acid is allowed to rise too high, the lactic acid bacteria will be killed. Then after autolysis of the dead bacterial cells, their proteolytic endo-enzymes will become active and possibly give rise to bad flavours in the butter.

BACTERIA IN BUTTER

Butter consists mainly of butter-fat, which is not readily attacked by micro-organisms. The types and numbers of bacteria in butter thus depend on the proportions of other substances, that is, on the amount of butter-fat finally left in the butter. In well-washed and worked butter, the numbers of bacteria increase after churning, but later decrease as the butter-milk is gradually used up. The lactic acid bacteria die off first as soon as the lactose has all been used and later the sporing bacteria. Newly-made butter from ripened cream usually contains between 10 and 20 million bacteria per gram, but after a period of storage, this should drop to about 100,000.

The keeping-quality of butter is influenced more by the types of bacteria than by their numbers. Butter from pasteurised, ripened cream contains mainly lactic streptococci, but that from raw cream will contain the types of micro-organisms present in the original milk. These may include lactic streptococci and lactobacilli, coliform bacteria, *Pseudomonas fluorescens*, yeasts and moulds. Such butter may also contain sporing bacteria which are very liable to cause deterioration during storage. The keeping-quality of butter is influenced by the following factors :—

(1) The amount of butter-milk left in the butter, as this is about the only source of food for bacteria.

(2) The dispersion of the water globules. If these are in the form of many small droplets, some are bound to be sterile thus reducing the amount of food available to the bacteria.

(3) The quality of the washing water, which if dirty will contaminate the butter. The good effects of efficient pasteurisation and

pening can be completely nullified if the water used to wash the utter is contaminated with bacteria, especially if those are putre-ctive types.

(4) Salting tends to kill or inhibit bacteria. A low percentage of lt in the butter can mean a high concentration of brine in the water roplets. Butter should legally have not more than 16% of water nd all the salt finally dissolves in this water. The high concentration f brine has an osmotic pressure too high for the bacteria, which are eriously inhibited, if not actually killed.

(5) The quality of the original milk plays a large part in deter-ining the final keeping-quality of the butter. Even thorough pasteur-ation will not kill spore-forming organisms and these will persist in ne butter and be a potential source of deterioration and bad flavours uring storage.

(6) The degree of acidity produced during ripening, if sufficient, ill kill off the putrefactive bacteria. In pasteurised cream, how-ver, ripening will not kill bacteria which have survived the heat-eatment.

ACTERIAL TAINTS IN BUTTER.

The most frequent types of deterioration and objectionable bad avours in butter due to micro-organisms are as follows :—

Rancidity, through splitting the butter-fat and liberating butyric id, sometimes produces a highly objectionable odour and taste. ancidity is caused by the fat-splitting enzyme, *lipase*. This is pro-uced by certain bacteria and fungi. Bacteria causing rancidity include *eudomonas fluorescens* and *Serratia marcescens* ,while moulds of this pe are *Oidium lactis*, *Cladosporium butyri* and *Penicillium glaucum*. onditions favouring the growth of these micro-organisms are a high roportion of butter-milk in the butter, high acidity, a high storage mperature, the presence of oxygen and the presence of light. Control easures for rancidity are as follows :—

(1) Thorough washing of the butter to eliminate all traces of utter-milk.

(2) Thorough working of the butter to eliminate surplus moisture nd to disperse the remainder as widely as possible. These two factors e intrinsic parts of good butter-making technique and both aim at ndering the growth of micro-organisms.

(3) Firm and close packing of the butter to exclude oxygen.

(4) Covering the surface of the butter to protect it from air and ght.

(5) Storing the butter in a cold, dry and dark place.

Bitter flavours in butter are caused by putrefactive bacteria such *Pseudomonas fluorescens* and *Bacillus mycoides*. These usually break own the casein before churning into bitter substances especially low temperatures have caused slow ripening. If the causal bacteria e bacilli, pasteurisation will not kill their spores so that the remedy r bitterness lies in producing clean milk not contaminated with these

bacteria. Also, if the cream is being ripened, a vigorous starter should be used and ripening carried out at a suitable temperature, such as 70° F, to encourage quick and active lactic acid production. This will inhibit putrefactive bacteria.

Putrid taint in butter is due either to the milk or washing water being contaminated with putrefactive bacteria such as *Pseudomonas fluorescens* and aerobic sporing bacteria like *Bacillus subtilis* and *B. mycoides*. These bacteria attack the milk proteins present in the traces of butter-milk therefore this taint is related to bitterness. The fault is favoured by a high percentage of residual butter-milk in the butter. Control of putrid taint involves the use of clean, uncontaminated milk and washing water in order to eliminate the putrefactive bacteria.

Fishiness is an exceptionally objectionable fault and most frequently occurs in imported butters. It may be produced by the mould, *Oidium lactis* or by *Bacterium ichthyosmius*. Occasionally yeasts may bring about fishiness if they are growing in association with lactic streptococci or lactobacilli. Fishy flavour is due to the production of trimethylamine from the lecithin naturally present in the milk.

Oiliness used to be a common complaint against imported Danish butter. It may be caused by lactic acid bacteria curdling the casein in the residual butter-milk and producing an oily flavour. Certain fat-splitting bacteria can also produce oiliness by hydrolysing the butter-fat.

"*Animal*" or *cowshed flavour* in butter is caused by heavy contamination of the original milk or washing water with coliform bacteria, the source of which will probably be dung. The use of clean milk and washing water will help to control this defect. Pasteurisation of the cream will kill off all coliform bacteria.

"*Turnipy*" *flavour* in butter may often be due to a food taint in the original milk, but it may also be caused by certain bacteria. In the latter case it will develop during storage. Taints due to the cow's food always tend to disappear during storage.

Various colour faults occasionally arise in bulk butter due to the growth of moulds on the surface. A black or pink discolouration is frequently due to the growth of torula yeasts, which usually also produce a yeasty flavour in the butter.

Pathogenic Bacteria in Butter.

Certain diseases, including tuberculosis and foot-and-mouth disease, may be passed on from the milk into the butter, if the cream has not been pasteurised. Pathogenic bacteria may also gain access to the butter from human sources, as for instance, when the dairy personnel are disease carriers. The use of contaminated water to wash the butter granules in the churn is another common source of disease in butter, especially of typhoid fever. Pathogenic bacteria may remain alive in butter for months especially where infection is introduced by post-pasteurisation contamination. Precautions to guard

against the introduction of pathogenic bacteria into butter include the following :—

(1) Pasteurisation of the cream to kill any disease producing bacteria.

(2) The cream should be well-ripened if it is not pasteurised. The vigorous production of lactic acid will kill or inhibit any pathogenic bacteria present.

(3) The supply of water used should be pure and free from sewage contamination.

(4) The milk, cream and butter together with all the equipment used, should be protected from flies, which are notorious carriers of disease.

(5) The dairy personnel should be healthy and free from all infectious diseases. Carriers of any disease should not be allowed to handle milk or milk products. Infected workers coughing over butter is a sure way to render it dangerous.

Butter should never be made from the milk of a cow suffering from mastitis as such milk is unfit for consumption, does not churn well and is liable to develop taints due to the possible presence of *Streptococcus agalactiae*, the principal type of mastitis bacteria.

BACTERIA AND MOULDS IN CHEESE-MAKING

Cheese-making is a biological process depending primarily on the activity of bacteria and fungi and their enzymes. As cheese contains about 33% of protein, it is readily attacked by proteolytic bacteria. The lactic acid in cheese checks these putrefactive organisms, but lactic acid alone will not prevent the growth of yeasts and moulds in the cheese. In hard-pressed cheese, these are inhibited by lack of oxygen. The various varieties of cheese are obtained by encouraging different types of micro-organisms.

There are two main types of cheese :—

(1) Acid-curdled and (2) Rennet-curdled.

Acid-curdled cheese includes those kinds in which the milk is coagulated by the action of lactic acid. The high acidity prevents the growth of many types of bacteria, but *aciduric* bacteria, that is, those types which can withstand acid conditions, can grow and are encouraged by the high moisture content of the acid-curdled cheese. Moulds grow very well on such cheeses as they are suited to the moist acid conditions of the loosely-packed curd, which also provides the moulds with an adequate supply of air. Cheeses of this type, including cream cheeses and the sour milk cheeses or "crowdies," have thus a very short life and must be consumed soon after making. In their manufacture the milk or cream is curdled by ripening with a vigorous starter of lactic acid bacteria. If ripening is allowed to continue too long so that the percentage of lactic acid in the milk becomes too high, the lactic acid organisms will be killed and autolysis will release proteolytic endo-enzymes from the bacterial cells. These enzymes then attack the cheese proteins and cause decomposition. As has been

noted, this also occurs in butter made from over-ripe cream. Coliform bacteria are acid-resistant and often produce gas in soft, acid-curdled cheese.

Rennet-curdled cheeses constitute a much wider group. They may be *hard*, *semi-hard* or *soft* according to the amount of moisture left in the curd. Examples of these types are Cheddar, Stilton and Camembert respectively. In hard cheese, ripening takes place either without oxygen or in the presence of very small amounts, that is, the ripening of hard cheese is either *anaerobic* or *micro-aerophilic*. In soft cheese, ripening is brought about by *aerobic* micro-organisms living on the surface and proceeds inwards from the outside.

Milk intended for cheese-making must be bacteriologically clean. In most large cheese factories, it is flash-pasteurised to kill off bacteria. The usual types of bacteria present in milk for cheese-making include lactic streptococci, coliform bacteria, various micrococci, sporing bacilli and some bacteria producing pigments. Yeast and mould spores may also be present in the milk. With rennet coagulation, preliminary ripening of the milk is essential as the acidity increases the activity of the rennin and pepsin enzymes in the rennet. This results in a firmer and quicker coagulation of curd. The initial ripening is done by inoculating the milk with about 1% of starter, usually a culture of *Streptococcus lactis* and *S. cremoris* in milk previously sterilised. Flash-pasteurising kills off all the bacteria originally in the milk except sporing types whose spores are resistant. Thus by adding starter, the two types of lactic bacteria provided by it become predominant. If other bacteria find their way into the milk, they are usually held in check by the vigorous starter bacteria. Old rennet may be the source of such extraneous organisms.

When the milk has ripened sufficiently to receive the rennet, it usually contains about 50 million bacteria per millilitre. These should consist almost entirely of lactic streptococci and lactobacilli. If the milk has not been pasteurised and the starter used is weak, some coliform bacteria and micrococci may also be present. Those micrococci which existed as udder commensals may prove beneficial by assisting in ripening the cheese after the curd has been pressed. They are *acido-proteolytic* which means that they can bring about proteolysis in an acid medium and so will hydrolyse the casein slightly. When the milk has coagulated sufficiently and the curd is cut, most of the bacteria remain in the latter and cause a great increase in its acidity during the later stages of cheese-making.

Bacteria and Moulds in Cheese Ripening.

The numbers of bacteria in the curd rapidly increase in the first few days up to about 1,000 million per gram and then, as ripening proceeds, there is a decrease which is rapid at first but slows down later. In fully ripe Cheddar cheese, the numbers of bacteria should not be more than between two and three million per gram. These bacteria are mostly lactobacilli. At this stage the lactose in the cheese has been almost wholly converted into lactic acid.

The rate of increase of bacteria in the curd, the stage of maximum numbers, the actual numbers and the rate of decrease all vary. The variation depends chiefly on temperature but also to a considerable extent on the supply of moisture and lactose in the curd. Growth of bacteria and, in consequence, ripening is retarded by low temperatures. In ripe cheese, the bacteria exist as colonies known as "vitamin centres" and many of these are pure cultures. The inter-spaces between the bacterial colonies may be completely sterile.

The types of bacteria to be found in ripe cheese also vary. *Streptococcus lactis* and *S. cremoris* are predominant in the original milk and in the starter and make up about 99% of the total bacteria in the fresh curd. They are responsible for the rapid increase in numbers during the first few days. As the percentage of lactic acid increases, these lactic streptococci die off. In fully ripe cheese there are very few of them. As the lactic streptococci die off, various lactobacilli develop and increase their numbers because they thrive in acid conditions. A few lactobacilli from fresh foods, silage, and the atmosphere of the cowshed always occur in raw milk. They are relatively slow-growing and can start to increase only after the lactic streptococci have died off. Lactobacilli are acid-tolerant and can easily withstand the acidity produced by the lactic streptococci. Their growth is later encouraged by the production of peptone from the casein as a result of enzymic proteolysis activated by the death of the lactic streptococci. Thus in cheese up to 14 days old, over 99% of the bacteria present are lactic streptococci and less than 1% are lactobacilli. In cheese six months old or more, the reverse is true.

Besides these true lactic acid bacteria, there are others commonly present in cheese. Spore-forming bacilli and *Pseudomonas fluorescens* frequently occur, but as they are not acid tolerant, they are usually killed off by the acid conditions prevailing in the fresh curd. If, however, the original milk was heavily contaminated with these types, then they may later cause taints in the cheese. Coliform bacteria may also be present in cheese. They are acid-tolerant and if present in large numbers may produce a taint and cause gassiness. Micrococci present in the cheese are not very important, but they may alter the flavour slightly by producing volatile substances.

After the death of the lactic streptococci, their endo-enzymes break down the peptones further into amino-acids and even free ammonia and its salts. After the lactic bacteria have used up all the lactose, some lactic acid may be produced from the casein.

Streptococcus citrovorus and *S. paracitrovorus*, the "pseudo-lactic acid bacteria," may have been present in the starter. During cheese ripening they will further attack the lactic and citric acids and convert them into various alcohols and esters which will flavour the cheese. Fat-splitting bacteria may also produce these flavouring substances from the fat in the cheese.

While lactic acid bacteria are the most important micro-organisms concerned in the ripening of hard-pressed cheese, the final flavour of the cheese depends primarily on the type of cheese-making process

used. This is turn mainly determines the types and numbers of bacteria which will be active in the ripening cheese.

SUMMARY OF CHEESE RIPENING.

FAT. ——→ Fatty acids and glycerol by a few fat splitting bacteria.
FAT. ——→ Fatty acids, esters and alcohols by lactic streptococci and various micrococci.

LACTOSE. ——→ Lactic acid, lactates and citrates by lactic streptococci and lactobacilli.

CASEIN ——→ Peptones by rennet enzymes and perhaps natural milk galactase.
CASEIN ——→ Peptones by enzymes of lactic streptococci, lactobacilli, and several micrococci.

FAULTS IN HARD-PRESSED CHEESE DUE TO MICRO-ORGANISMS

It is not sufficient that milk for cheese-making should have a low bacterial count. If all the bacteria present consist of lactic streptococci and lactobacilli, then large numbers are advantageous. On the other hand, if the milk has been contaminated with coliform bacteria, then the number of bacteria in the milk should be as small as possible. Faults in hand-pressed cheese may be due to heavy contamination of the original milk with one type of organism, but are more frequently caused by the increased growth of that type due to abnormal conditions in the cheese. Thus in milk destined for cheese-making, the total number of bacteria is not nearly so important as their types. A frequent cause of faults is the use of a weak starter. In this case the development of lactic acid during the initial ripening in the vat is slow so that the growth of any putrefactive bacteria present in the milk is not effectively checked. These bacteria remain alive and active in the curd and may produce a taint or poor texture in the final product.

There are faults in hand-pressed cheese due to specific types of micro-organisms :

Gassiness is a fault which honeycombs the curd or ripe cheese with thousands of holes. It is caused mainly by coliform bacteria but may also arise through the activities of yeasts or of anaerobic spore-forming clostridia. Normally cheese contains less than 0.5% of carbon dioxide, but any of the above micro-organisms may ferment the lactose and produce additional amounts of this gas. If the gas is produced while the curd is still in the vat, the curd may float and prove very difficult to " pitch." It does not shrink normally and express the whey as it should. This type of curd is known as " pin-hole " curd. If gassiness arises during ripening and after the curd has been pressed, the texture becomes spongy and the cheese swells into domes at the ends. The gas may be released by puncturing the cheese, but this allows air to enter and spoils the texture.

Discolouration of cheese is usually caused by bacteria which can produce pigments. If the pigment is soluble in water, it

will diffuse through the cheese. If it is not, it will be found in the form of patches. Fat-splitting bacteria can cause mottling and bleaching, but this may also be due to excessive acidity or uneven moisture distribution. Proteolytic bacteria are also capable of causing discolouration; they usually produce unpleasant flavours at the same time. *Rusty spot* is a fault in which, on cutting the cheese, rusty coloured spots are found dispersed throughout. It is caused by certain lactobacilli and also by an organism known as *Serratia marcescens* or *Bacterium prodigiosus*, which can survive pasteurisation. The rusty spots take about one week to develop and occur only in summer as the causal bacteria originally come from grass. *Pink patches* scattered throughout the cheese arise owing to the reduction of annatto by various moulds. This trouble occurs only when annatto is being used, that is, mainly in the early spring. Colours in cheese due to putrefactive organisms include a *blue discolouration* sometimes caused by *Pseudomonas syncyanae* and a *black surface discolouration* due to the growth of moulds on the outside of the cheese. A *grey blotchiness* throughout the cheese is not due to micro-organisms, but is caused by the presence of traces of dissolved copper and iron in the original milk. These metals are derived from worn utensils, such as milk coolers, and act as catalysts assisting undesirable reactions in cheese and other dairy products. Surface discolourations on cheese are very unsightly, but they are not serious as they are easily removed.

Bad Flavours in Cheese.

These may be caused by coliform bacteria, mastitis organisms, yeasts or proteolytic bacteria and all appear during ripening. The responsible micro-organisms tend to neutralise the developed acidity. Moreover, any bad flavour in the milk may persist in the cheese. A *fruity, sweet flavour* can arise through the growth of yeasts or the mould, *Cladosporium*. A *cabbage flavour* may be due to the mould, *Penicillium* and tends to be worse in soft cheeses. A *putrid flavour* and " bad smell " are caused by anaerobic spore-forming clostridia. These bacteria usually gain access to the original milk from dung, but occasionally also from rennet. *Bitter flavours* in cheese are usually caused by yeasts and proteolytic bacteria such as *Streprococcus liquefaciens*. Milk from a cow with mastitis may also produce undesirable flavours in the ripe cheese. A *malty* or *burnt flavour* is caused by *Streptococcus lactis maltigenes* which may be associated with its near relatives, the lactic streptococci. A *cowshed* or *animal taint* in ripe cheese is usually caused by heavy contamination with coliform bacteria. A *tallowy flavour* is not usually biological in origin, but is due to catalytic oxidation of the fat as in the case of the grey discolouration.

All the above micro-biological faults and taints in cheese may be effectively controlled by attention to the following recommendations:—

(1) Clean milk should always be used for cheese-making, and should be thoroughly and quickly ripened with a vigorous starter.

(2) The previous evening s milk, if used for the next day's cheese, should be properly cooled before it is left overnight in the vat.

(3) The correct degree of acidity should always be attained during each stage of the making process. As well as ensuring a high quality of cheese, this will give maximum control of any undesirable micro-organisms.

(4) The correct rate of salting should be used before the curd is packed into chisets. This improves the flavour and texture of the cheese. It also assists in the inhibition of undesirable bacteria by exerting considerable osmotic pressure in the residual whey in the curd.

(5) All utensils and equipment should be thoroughly sterilised or scalded before coming into contact with the milk or curd. This eliminates any risk of contamination with bacteria from any equipment used during cheese-making.

(6) In order to encourage the desirable types of bacterial growth and therefore the correct type of ripening, the ripening room should be kept at a suitable temperature and humidity.

Mould Growth on Hard-Pressed Cheese.

Usually moulds can grow only on the outsides of hard-pressed cheeses but they can cause cracking of the rind. They enter the cracks and penetrate right into the body of the cheese. Once inside, they hydrolyse the fat and so give rise to off-flavours. The growth of moulds on hard-pressed cheese may conveniently be inhibited by the following measures :—

(1) The correct degree of humidity should be maintained in the ripening room. If the atmosphere of this room is too moist, moulds will grow readily. If, on the other hand, it is too dry, the cheese rinds will tend to crack.

(2) Mould spores are invariably to be found on the shelves of the ripening room. These can easily be killed by scrubbing the shelves with a weak solution of formalin, sodium hypochlorite or calcium chloride.

(3) The cheeses may be " paraffined," that is, they may be dipped into hot paraffin wax, which will inhibit mould growth and also prevent the cheeses cracking through loss of moisture.

MICRO-ORGANISMS IN CONDENSED MILK

Condensed milk is preserved against deterioration through bacteria and fungi, by the removal of moisture followed by the addition of sugar to produce a high osmotic pressure. The product is then sealed in tins; this excludes bacteria. Condensed milk is never sterile but the growth of micro-organisms is inhibited by the high osmotic pressure. Even after pre-heating to 200°F and condensing under partial vacuum at 140°F, it usually contains from 100 to 100,000 micro-organisms per gram. The commonest types of these are micrococci, staphylococci and other pigment-producing bacteria. Aerobic spore-forming bacilli and torula yeasts may also be found, but they become active and cause trouble only if air gains access to the tin.

Faults in Condensed Milk Caused by Micro-Organisms.

These faults depend principally on the type of microbial contamination of the original milk. The commonest are as follows :—

Gassiness can easily be detected by the occurrence of "blown" tins which bulge out at the ends. This fault may be caused both by yeasts and by coliform bacteria. These organisms ferment the added cane or beet sugar with the production of gas. Yeasts produce "yeasty" off-flavours as well as gas. As coliform bacteria are not heat-resistant, contamination must have occurred after condensing. Usually coliform bacteria gain entrance during cooling and handling prior to canning. Whole batches of tins not infrequently "blow" at once thus indicating that the contamination has been general. Yeast spores can survive the condensing of milk; if faulty sealing of the tins permits oxygen to gain access to the contents, yeasts may be active in causing gassiness. Infestation of the condensery with flies and other insects or dirty and insanitary working conditions may also be responsible for an outbreak of gassiness in tins of condensed milk.

Thickening of the condensed milk in the tins is not so obvious as gassiness. It can, however, be detected by shaking the tins when the affected ones will emit a more "solid" sound than those still normal. Thickening may be local and confined to parts of the tin, or it may be general throughout the contents. It is a bacterial fault and is caused by various cocci which produce acids able to coagulate the milk proteins partially. Some bacteria secrete a rennin-like enzyme which will coagulate casein and so bring about thickening. Off-flavours are nearly always found to accompany thickening. Both these faults can be prevented by using clean milk. Thickening may also be physical in nature due to a long period of storage, but in this case, there are no associated off-flavours.

The occurrence of *buttons* or *lumps* is a fault caused by several fungi. The lumps occur on the surface and can often be picked out easily. The causal fungi secrete enzymes which cause local coagulation of the milk proteins. These fungi require oxygen in order to grow; thus their presence in condensed milk points to faulty sealing of the tins and the control of "buttons" lies in sealing under vacuum. Low storage temperatures also prevent rapid mould growth.

MICRO-ORGANISMS IN EVAPORATED MILK

In the manufacture of evaporated milk, preservation is effected by condensing in a vacuum pan without adding sugar, but the product is sterilised in the tins after sealing. Evaporated milk provides an excellent medium for the growth of any bacteria which have escaped destruction. Usually, sample tins are incubated at 22° C and at 37° C after sterilisation in order to detect contamination and subsequent faults. The commonest bacterial faults in evaporated milk are described below.

Coagulation may be caused by two types of bacteria. Lactic streptococci may attack the lactose and produce lactic acid. If this goes far enough, the casein is coagulated. As lactic streptoccoci are far from being heat-resistant, their occurrence in evaporated milk

indicates gross contamination after sterilising and the sealing of the tins must be faulty. This type of coagulation is usually restricted to certain tins. The second type of coagulation is caused by the growth of " sweet-curdling " bacteria. These are mainly aerobic spore-forming bacilli; they secrete an enzyme called " protease " which can cause coagulation of the milk proteins. Their spores are very resistant to heat and are not killed by sterilisation, especially if this is inefficiently carried out. Coagulation may, like thickening in condensed milk, be purely physical in origin and brought about by sterilising at too high a temperature or storing the tins in a warm place.

Gassiness resulting in " blown " tins occurs in evaporated milk also. It may be caused by coliform bacteria, certain cocci or anaerobic spore-forming clostridia. All these bacteria can ferment lactose with the production of carbon dioxide gas. If the fault is due to the growth of coliform organisms or cocci, contamination must have occurred after sterilisation as these types are not heat-resistant. Faulty sealing of tins often leads to this type of gassiness. On the other hand, the spores of clostridia are highly heat-resistant and may not be killed during sterilisation. If this type of gassiness arises, the original milk must have been contaminated with these sporing bacteria. As clostridia are anaerobic and can grow without free oxygen, perfect sealing will not prevent their multiplication. Gassiness in evaporated milk is always associated with taints and off-flavours. It occasionally arises as the result of a purely chemical reaction between the metal of the tin and certain acids in the milk. For this reason, the interior of the tins are usually treated with a special lacquer to protect the metal from the action of the milk acids.

Bitter flavours may or may not be associated with gassiness and coagulation. They are usually caused by sporing bacilli which have remained active as a result of faulty sterilising and faulty sealing.

Fishiness in evaporated milk, as in butter, is caused by a micro-organism called *Proteus ichthyosmius* and is usually associated with coagulation and proteolysis.

Efficient sterilisation should destroy all bacteria except the spores of bacilli and clostridia. In clean milk these spores should be too few in number to cause much trouble.

MICRO-ORGANISMS IN MILK POWDERS.

Milk powders are preserved by the removal of water, as bacteria cannot grow when the moisture content is reduced below a certain critical figure. Dried milk is, however, never sterile. Bacteria in dried milk consist of the survivors of the drying process and any contaminants which may later gain access. Neither of these types should develop and multiply during storage. Roller-drying employs higher temperatures than spray-drying and kills bacteria more effectively. The average number of bacteria in milk powders is between 300 and 1,000 per gram, but post-drying contamination during packing and other handling operations may cause an increase to about 5,000. The bacteria found in milk powder immediately after drying are mainly heat-

resistant non-sporing types. Spores may also occur but these are infrequent in normal clean milk. Bacteria contaminating the milk powder after drying are chiefly cocci. Frequently yeast and mould spores are also present.

Most of the micro-organisms in milk powder gradually die off during storage due to lack of moisture. The number decreases and is then maintained at a constant low level. Thus the deterioration of milk powders is not usually brought about through the agency of micro-organisms. If milk powder becomes damp, the first types to grow are moulds.

Faults in Milk Powders.

Rancidity may occur during storage at high temperatures due to the action of a natural milk lipase. This enzyme, especially in the presence of traces of copper, attacks the fat and hydrolyses it into free fatty acids and glycerol. This lipase is usually destroyed during the drying process, but occasionally may survive spray-drying. *Tallowiness* is due to the oxidation of the fat. The reaction is catalysed by the presence of traces of copper and iron in the milk powder. *Staleness* is an insidious type of deterioration in which there is a decrease in solubility and a gradual darkening in colour. It is due to an increase in the moisture content of the milk powder.

BACTERIA IN ICE-CREAM

Ice-cream may contain large numbers of bacteria, and if it is manufactured under unhygienic conditions, pathogenic types may be present. Freezing and storage at low temperatures will not destroy these micro-organisms. Ice-cream may be a source of outbreaks of certain diseases, such as typhoid and dysentery, and, as such, it is second in importance only to raw milk. Most of the bacteria in ice-cream are of the lactic acid or pseudo-lactic acid type, but if the numbers are very high, there is usually a much wider range of groups. There may be one predominant species due to a single heavy initial contamination. Such predominant species are frequently coliform bacteria or one of the sporing bacilli. The sources of bacteria in ice-cream vary widely in their importance and include the following :—

(1) The *raw materials*, that is, the constituents of the ice-cream mix. The most important of these is the milk or milk solids used. Ice-cream may contain any of the bacteria which were in the original milk. The milk, separated milk or cream should be pasteurised and stored at a low temperature before use. Cream cannot at present be employed. Pasteurisation followed by cold storage reduces considerably the number of bacteria in the product. The extent of initial contamination from the raw materials depends obviously on their bacteriological quality. Gelatine is widely used as a stabiliser in ice-cream. If it is stored under dry conditions, it contains few bacteria. If, however, it becomes damp in storage, it may become contaminated with putrefactive bacteria. When gelatine is used, it must first be dissolved by heat. This will kill many of the bacteria in it, except

for putrefactive sporing bacteria and some micro-cocci. The sugar usually contains very few bacteria, but it may contain a few yeasts or may have been contaminated by flies. The flavouring may also contain yeasts and mould spores, especially if it is a fruit juice. Synthetic flavourings are usually sterile.

(2) *Utensils* are the worst source of bacteria in ice-cream. Both the homogeniser and freezer are very difficult to sterilise effectively and may add large numbers of organisms to the product. They should be sterilised immediately before use either by blowing steam through the equipment or by running boiling water through.

(3) The *personnel* in the factory are, as with all milk production, liable to be carriers or sub-clinical cases of diseases and may infect the ice-cream. Such persons should be strictly forbidden to handle ice-cream.

(4) *Bacteria from the atmosphere* may gain access to ice-cream. This form of contamination is worst with open tub freezers in hot dusty weather. In modern enclosed freezing plants air contamination is almost negligible.

In view of the possibility of ice-cream being a vehicle for the spread of disease, prescribed heat-treatments must be carried out under the Ice-Cream (Heat-Treatment) Regulations which came into force on May 1st, 1947. These regulations require that :—

(1) Ice-cream mixes prepared in the place of sale must be pasteurised at either 150° F for 30 minutes or at 160° F for 10 minutes. They must also be cooled afterwards to 45° F within one and a half hours. This does not apply to ready-made cold mix powders.

(2) If the ice-cream is at a temperature of over 28° F after freezing, it must be re-pasteurised by one of the methods described earlier.

Pathogenic Bacteria in Dairy Produce.

Butter may contain living pathogenic bacteria as they are not always killed off by the acidity developed during the ripening process. Even if all these types are killed by pasteurisation of the cream, more may gain access from dairy workers who are infected or who are carriers of a certain disease. Contaminated utensils have also been known to introduce disease-producing bacteria into a batch of butter. It has been proved that certain types of pathogenic bacteria can exist in the butter even in cold storage for several months.

In cheese, the acidity developed during the manufacturing process tends to destroy disease-producing organisms. In addition the milk is often flash pasteurised to kill off bacteria. Despite this, it is possible for cheese to harbour certain diseases derived from workers who themselves were infected or who were acting as carriers.

Ice-cream is a very common source of disease outbreaks as any of its constituents, namely, milk, sugar, gelatine, flavouring, etc., may be contaminated with pathogenic bacteria. Freezing does not kill bacteria. The gelatin is frequently contaminated from non-sterile

utensils, flies and from human sources. It is responsible for some of the most undesirable bacteria in ice-cream. Typhoid and paratyphoid fevers are two diseases which are frequently spread through the agency of ice-cream. A recent example of this was the outbreak of fever at Aberystwyth in the summer of 1946.

Poisoning from Dairy Produce.

Cases of toxic poisoning occasionally arise from milk products, especially from cheese. The toxins themselves are often never identified, but *Clostridium botulinus* may be the cause in certain cases. This micro-organism lives in the soil and may also be found in the faeces of poisoned animals and humans. Its spores are highly resistant and are not destroyed by pasteurisation while the actual toxin itself is not altered even by continual boiling. The condition of poisoning from this organism is known as "botulism."

Poisoning from milk products may be non-bacterial in origin. If the cow is upset emotionally or is suffering from certain diseases, she may secrete toxic substances in the milk. Persons consuming such milk, or products derived from it, may suffer from digestive upsets. Various drugs, including some of the new sulpho drugs, are eliminated from the cow's body in her milk and these may also upset certain people. In the United States of America, there is a condition among humans known as "milk sickness." This is a form of poisoning contracted by drinking the milk of cows which have been feeding upon a certain weed. This weed is apparently poisonous to humans and yet has no effect on the cows. The toxic principle is however, passed on in the milk, so causing the illness in the consumers.

The presence of dissolved metals such as copper and iron, may lead to changes in milk and its products which can cause indigestion in humans. This mild form of poisoning is at its worst when warm acid milk is run over a badly-worn cooler. Poisoning in dairy produce ranges in acuteness from a mild degree of indigestion, due perhaps to an off-flavour in the product, to a fatal case of botulism.

SECTION III.—THE BACTERIOLOGICAL TESTING OF MILK.

SAMPLING OF MILK FOR BACTERIOLOGICAL EXAMINATION.

Any one of the methods detailed for the drawing of milk samples for chemical tests may be used for samples intended for bacterial tests. While it is not so fundamentally important for a bacteriological sample to be fully representative of the bulk milk as it is for a chemical sample, the bulk milk should be well mixed to distribute the bacteria. If a churn of milk is left standing for some time, many of the micro-

organisms are carried into the cream layer as they cling to the fat globules, while others adhere to any particles of solid dirt and sink to the bottom. It is only from a well-mixed bulk of milk, therefore, that a sample can be drawn which will show the true bacteriological quality of the whole. Of far greater importance than the mixing of the milk before sampling is the state of sterility of the equipment used. If the results of tests carried out on the sample are to be at all reliable, all bottles, dippers and tubes must be completely sterile, and must be used only once until they have been thoroughly re-sterilised. If any of these items of equipment have bacteria or their spores clinging to them, then these latter will be introduced into the milk giving apparently poorer results in the various tests. Equipment to be used for the bacteriological testing of dairy water-supplies and utensils must also be perfectly sterile. Glass sample bottles and metal plungers and dippers are best sterilised in a hot air oven at 180°C for two hours, while cotton and other fabric swabs may be sterilised by steaming them at 100°C for 30 minutes on each of three successive days. This latter method is known as " intermittent steam sterilisation".

TESTING OF MILK AND ITS PRODUCTS

Fresh milk is the perfect food for humans and also for many types of bacteria. If bacteria are allowed to grow in milk, then that milk may become not only unpalatable and repulsive in appearance, but highly dangerous to humans. If the nation's milk supply is to be both clean and safe, it must be tested regularly to eliminate those supplies which are contaminated.

There are many tests to which milk may be subjected. On the chemical side, these include tests for the percentage of butterfat, solids-not-fat, lactose, chlorides, acidity, phosphatase (for pasteurisation efficiency), etc. Among the bacteriological milk tests, there are the resazurin, methylene blue and coliform tests; plate counts and sediment tests; catalase tests and leucocyte counts for mastitis, and many more.

Tests to Determine the Keeping Quality of Milk.

The simplest test of this type is known as the *Keeping-quality test* and consists of keeping the milk sample at a constant temperature, usually room-temperature, until it sours. The length of time taken by the sample to turn sour is noted and, if necessary, recorded. It is far from being an accurate test, but its very simplicity renders it useful, not only for single samples, but for the comparison of various samples.

As clean milk should take several days to go sour, the test may be speeded up by incubating the samples at 22°C, which is the most suitable growth temperature for souring bacteria, such as *Streptococcus lactis*.

Dye-Reduction Milk Tests.

There are two of these tests and both are in wide use in creameries and milk-testing laboratories. They measure the keeping-quality

indirectly and are simple and accurate. They depend on the fact that souring bacteria, and also leucocytes, produce an enzyme called *reductase* which has the power of changing the colour of certain dyes by removing oxygen from them. The more souring bacteria there are in a sample of the milk, the more reductase there will be and at the same time the lower will be the keeping-quality. Thus the keeping-quality of the sample can be estimated by finding the amount of reductase in it.

The Methylene Blue or Reductase Test.

This is one of the tests based on the above principle. The dye used is "methylene blue" which reductase can change from blue to white; the rate of reduction—that is, removal of oxygen—is directly proportional to the keeping-quality. The test is carried out as follows :—

Pour 10 ml. of the milk sample into a sterile test-tube and add 1 ml. of standard methylene blue solution. This solution is made up by dissolving one tablet of methylene blue in 200 ml. of glass-distilled water and then making the solution up to 800 ml. with more glass-distilled water. The standard tablets of the dye are prepared by commercial firms and are readily obtainable. The stock solution of methylene blue should be sterilised in a steam steriliser before use. It may be stored for several weeks. All glassware used in the test should previously have been sterilised thoroughly.

Adding methylene blue to 10 ml. of milk in the tube.

Fit the tube containing 10 ml. milk and 1 ml. methylene blue solution with a rubber stopper previously sterilised in boiling water. Invert several times to mix the contents and place in a water-bath at 37°C. Invert the tube again at half-hourly intervals to disperse the cream and note the time taken for the dye to turn white. If the cream is allowed to rise, the bacteria, which produce the reductase, are carried up with the fat globules and the test is unreliable, as a white colour develops in the cream-line while the rest of the tube is still

blue. In the water-bath the tube must be kept in the dark as light also causes the blue colour to change to white. The result is recorded as the number of hours the tube takes to reduce, that is, to turn white.

Placing tube containing milk and dye in the water-bath.

This test is applied to Graded Milks as follows :—

T.T. and Accredited Milks. Take the sample the previous evening and hold overnight at room or laboratory temperature, or take a morning sample, hold during the day at room temperature and put in a refrigerator at 5 p.m. In both cases test at 10 a.m. next day. These milks should not decolourise the dye in 5.5 hours in winter or 4.5 hours in summer.

T.T. (*Pasteurised*) *and Pasteurised Milks.* Fill the test-tubes with milk, allow to stand overnight and add the methylene blue solution between 9 and 10 a.m. the next morning. The blue colour should last for at least half an hour.

(*Note.* Pasteurisation should kill off the souring bacteria and all others except the sporing types).

The Resazurin Test.

This is the same in principle as the methylene blue test, but the dye used is *resazurin*, which has the advantage that it is decolourised by reductase in stages through a range of colours from blue-purple to pink and finally to white. This occurs as the dye is reduced from resazurin to resorufin and finally to dehydro-resorufin. These colour changes allow of a more rapid result.

The actual technique of the test is very similar to that of the methylene blue test: 10 ml. of the milk sample are put into a sterile test-tube and 1 ml. of standard resazurin solution is added. This solution is prepared by dissolving one tablet of resazurin dye in 50 ml. of sterile glass-distilled water. Standard resazurin tablets are also manufactured commercially and are readily obtainable.

The tube is fitted with a sterile rubber stopper and incubated in a water-bath in the dark at 37° C for a definite time, usually one hour in the standard test or 10 minutes in the platform test. This is the main difference between the resazurin and methylene blue tests. The tube is then removed and its colour measured in the Lovibond comparator.

Diagram of a Lovibond Comparator

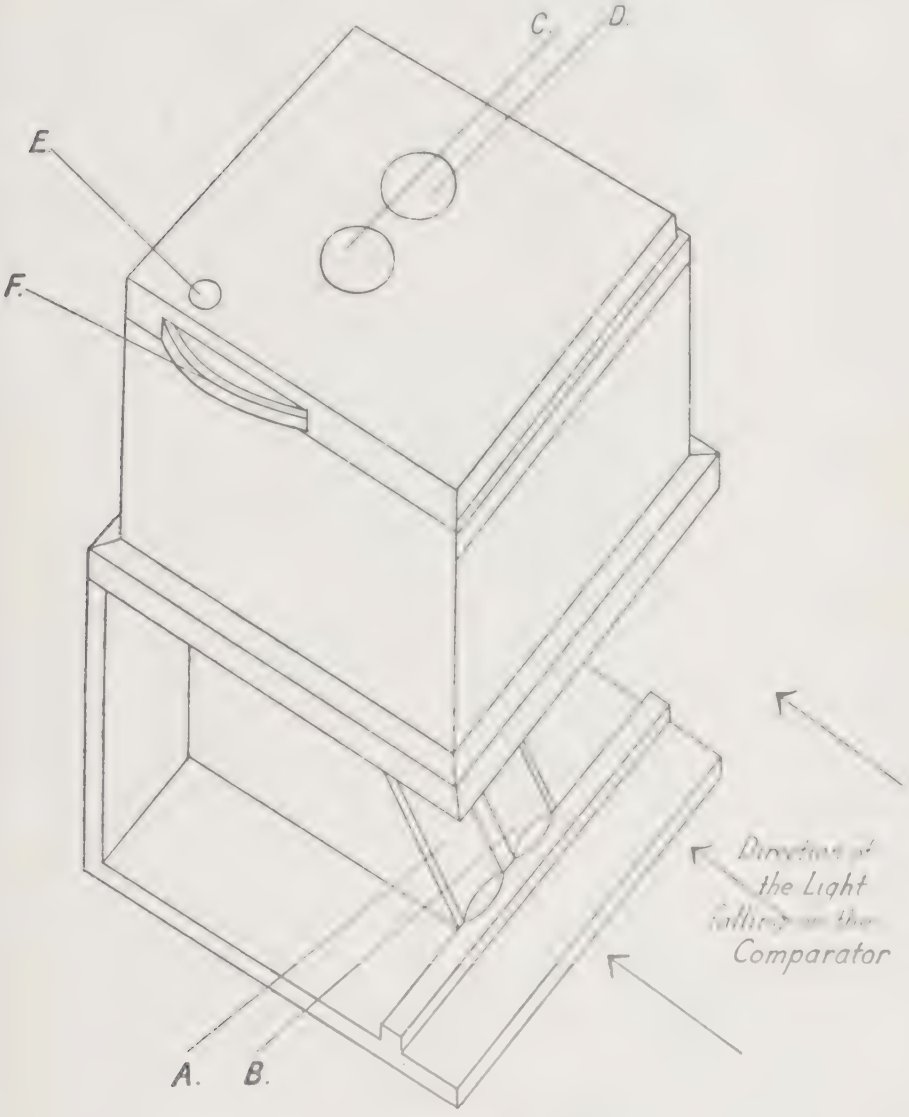


Fig. 44.

The use of this instrument allows the stage of reduction reached in the tube to be determined accurately. The Lovibond comparator is shown in *Fig.* 44, and in the accompanying photograph. It consists of a bakelite framework with spaces to hold two test-tubes at an inclination of about 50°. The incubated tube containing the milk and resazurin dye is placed on the right hand side at A, while a blank

control tube containing fresh milk of normal colour, is set on the left-hand side at B. On top of the comparator there are two view-holes. On looking down through these the operator can see the two tubes of milk side by side and is thus enabled to compare their colours. The milk tube under test is observed directly through C, but in the line of view through D to the control tube a piece of coloured glass is interposed. Six different colours can be placed in turn in front of the control tube by rotating the bakelite disc F. These colours range from the blue-purple colour of the pure resazurin solution through all its various stages of reduction to white. As each tint of glass is swung into position under the left-hand aperture, a number also comes into view in a small window E, set in the bottom right-hand corner of the top of the comparator. Number 6 appears when the blue-purple tinted glass is in position while number 0 is seen when the colourless or white glass is in place. These numbers are known as " resazurin disc numbers". The result of the test is expressed as a certain disc number.

Using the Lovibond Comparator in the Resazurin Test.

In order to find this disc number the incubated and control tubes are placed in their respective sides of the comparator. The operator looks down through the two apertures on the top of the comparator body, and with his right hand rotates the disc in which the tinted glasses are mounted until the glass whose colour matches that of the incubated tube comes into position. The number appearing in the small window is noted. This is the " resazurin disc number " of the sample.

Two modifications of the resazurin test are in wide use for grading milk supplies at dairies and creameries. The following is the grading system adopted by the National Milk Testing and Advisory Service.:—

Disc Number	*Grade*	*Disposal of Milk at Creamery*
4 or higher	A	Accepted by the creamery.
$3\frac{1}{2}$—1 (inclusive)	B	Salvaged for manufacture if possible.
$\frac{1}{2}$—0	C	Rejected and returned to producer.

Bulk samples of each producer's milk are taken on the creamery platform every 2 months, and the milk disposed of in accordance with the grading. The two modifications of the resazurin test used by the N.M.T. & A.S. are as follows :—

1. *The temperature-compensated resazurin test.* (*Standard Resazurin Test*).

This test is used for the routine testing of bulk samples at bi-monthly intervals. The time of incubation is determined by averaging the atmospheric maximum and minimum temperatures for the 24 hours preceding testing. Morning samples are held at atmospheric shade temperature and tested at 10 a.m. the next day. Evening samples are kept at shade temperature and tested at 4 p.m. the next day. The temperature during this period has a pronounced effect on the development of lactic acid bacteria in the milk and consequently on its keeping quality. To allow for these "weather differences" the period of incubation is fixed as follows :—

Average of Atmos. Max. and Min. °F	*Incubation Time at 37° C Minutes*
40 or below	120
40-50	90
50-60	60
60-65	30
65 and over	15

After the appropriate period of incubation, the tubes are removed and the disc numbers determined and used for grading as previously indicated.

2. *The Ten-Minute Platform Resazurin Test.*

This test is performed on single churns of doubtful odour and quality, or during a period of hot weather. The test is not necessarily performed in a milk-testing laboratory, but can be carried out on the creamery platform. The tubes are incubated for ten minutes only and graded from the resazurin disc numbers.

Comparison Between the Methylene Blue and Resazurin Tests.

Resazurin is much more sensitive than methylene blue to the reducing action of the reductase enzyme. For this reason the resazurin test is much the quicker. One hour's incubation with resazurin is equivalent to five hours' incubation with methylene blue. Resazurin is also much more sensitive to leucocytes than is methylene blue and will indicate milk from a cow suffering from mastitis much better than will methylene blue. This is a particularly important point if the milk is to be manufactured into cheese. Because of its characteristic property of reduction through various colour stages, resazurin provides much the more accurate measure of the keeping qualities of a milk sample.

The methylene blue test is, however, very easy to perform and demands very little skill. Also a stock solution of this dye may be stored for a considerable period, whereas resazurin solution has to be

prepared daily. On the other hand, it fails to detect milk from a cow infected with mastitis. Moreover, colostrum, early lactation and late lactation milks reduce the blue colour very quickly. This test consequently fails to differentiate between high grade normal milks of the above three types and milks from cows affected with mastitis. The methylene blue test is unreliable if the milk sample has previously been held at a very low temperature, as, for example, during frosty weather. During periods of frost, milks containing up to 500,000 bacteria per millilitre occasionally pass the methylene blue test.

Due to these disadvantages the methylene blue test is not now used for routine purposes. It is still employed for testing Graded and Designated Milks.

Control tubes containing milk without the dye are employed in both tests. Another type of control tube containing sterilised milk plus dye may also be used. The first type of control tube enables the operator to be sure that there is no impurity in the tube affecting the colour of the milk during incubation. The control tube containing the sterilised milk and dye will permit the detection of impurities in the stock solution of the dye affecting its colour during incubation. The presence of either of these kinds of impurities invalidates the results.

The Presumptive Coliform Test.

This is the universal test used to indicate faecal contamination of milk, water, and other liquids liable to be contaminated in this manner. It detects the presence of coliform bacteria in the milk or

Method of preparing Dilutions for the Presumptive Coniform Test

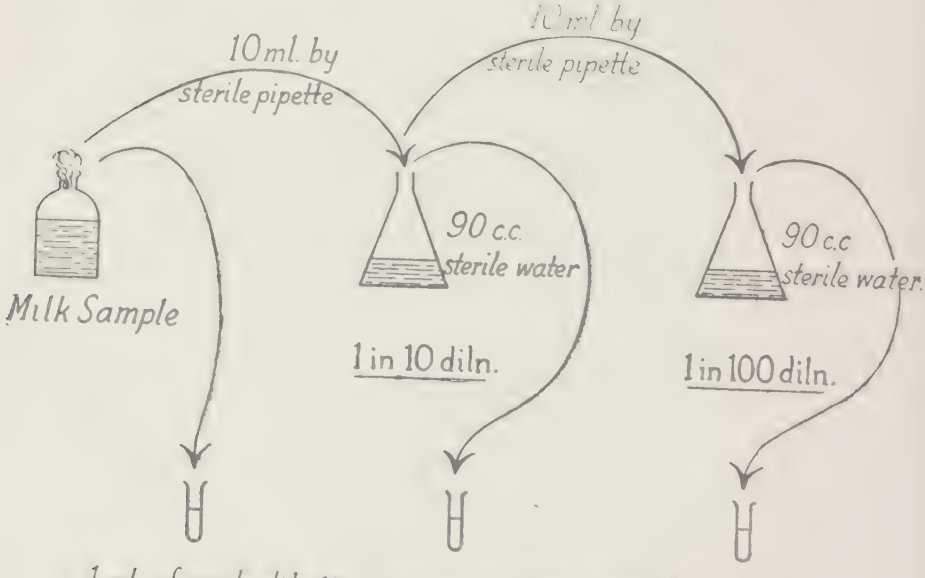


Fig 45.

water, and, in addition, determines how heavy is the degree of contamination. The milk to be tested is first mixed thoroughly. Sterile equipment is of the utmost necessity and the sample must be drawn under aseptic conditions.

Before testing, the sample is first diluted several times with either sterile water or sterile Ringer's solution. Ringer's solution is the more suitable of the two as its osmotic pressure is very close to that of the bacterial cells themselves, and it does not therefore tend to render any coliform bacteria in the milk inactive as quickly as does pure sterile distilled water. The milk sample is diluted before testing to allow the degree of contamination with coliform bacteria to be accurately estimated, for example, to find out if coliform types are present in 1 ml., 1/10th ml. or 1/100th ml. The usual dilutions are prepared according to the diagram.

Preparing dilutions for the Coliform Test or the Plate Count.

One millilitre of milk and of each dilution prepared is next inoculated into a "coliform tube" with a sterile pipette. These pipettes are used only once and are then discarded to be washed and re-sterilised The "coliform tube" used is simply an ordinary hard-glass test-tube containing several millilitres of a special liquid medium known as McKonkey's Bile Salt Broth. This medium is made up to the following recipe :—

Sodium glyco—or tauro-cholate (bile salt) ...	5 grams.
Peptone	20 grams.
Lactose	10 grams.
Water (ordinary tap)	1,000 ml.
Andrada's indicator or litmus	10 ml.

In addition to this liquid medium, a coliform tube contains a small inverted tube, known as a "Durham tube" which is placed in the liquid in order to trap any gas given off from the medium.

Diagram of a Coliform Tube

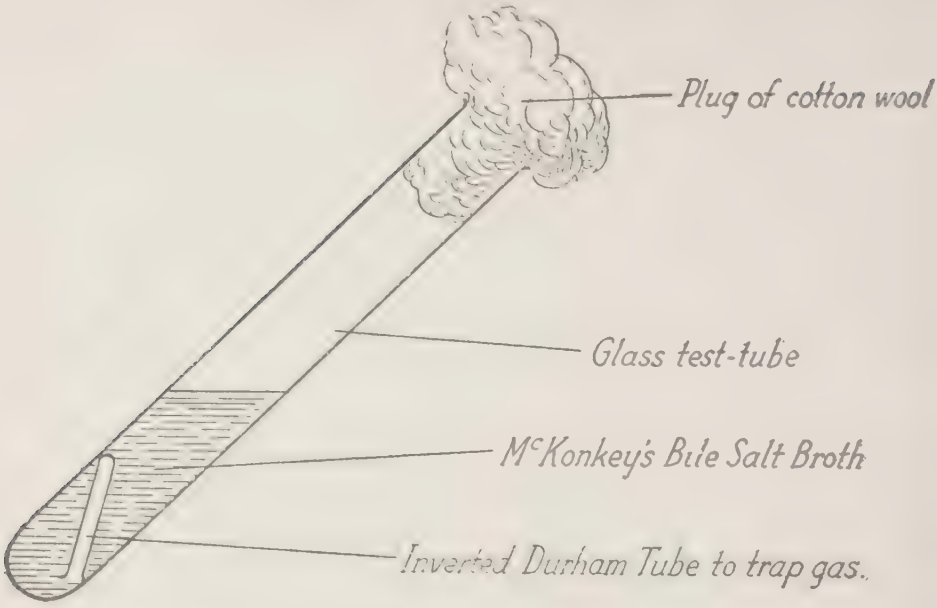


Fig. 46.

The principle behind the use of this bile-salt broth is that the bile-salt will inhibit all bacteria in the milk except coliform types. These coliform bacteria will thrive in the presence of bile-salt as they are natural inhabitants of the intestines of animals and are therefore, adapted to the presence of bile secretions. If any coliform bacteria are present in the milk, they will, on inoculation into the coliform tube, commence to ferment the lactose with the production of acid and the evolution of carbon dioxide and some hydrogen. The presence of developed acid may be noted by the indicator changing colour, while the gas bubbles will collect in the Durham tube.

Inoculating coliform tube with 1 ml. of milk or its dilution.

The tubes, each inoculated with 1 ml. of milk or its dilution, are next placed in an incubator and incubated at 37 C for 48 hours. This period of incubation at blood heat gives any coliform types present time to ferment the lactose and produce acid and gas. Lactic acid bacteria and other types will not grow due to the inhibiting action of the bile-salt.

After 48 hours incubation, the tubes are removed from the incubator and examined for acid and gas production. If coliform bacteria are present in the tube, then the indicator should have changed colour and the Durham tube should contain gas bubbles. Unless both acid and gas are present in the tube after incubation, coliform organisms are not assumed to be present in the tube, nor, therefore, in the 1 ml. of milk or its dilution originally added to the tube.

Placing coliform tubes in the incubator.

According to the greatest dilution which yields a positive result, that is, in which coliform have been detected, so the result is recorded. For instance, if three dilutions of 1 in 1, 1 in 10, and 1 in 100 were all tested and acid and gas were produced from the first two, then the result of the test on that particular milk sample would be stated as " coliform present in 1/10th ml. and absent in 1/100th".

This presumptive coliform test is used to check the standards of production of Tuberculin Tested and Accredited milks. In the case of these two Designated milks, there must be no coliform bacteria in 1/100th of a millilitre of milk.

The significance of the coliform test is not the mere presence of coliform bacteria in the milk, but the possibility of the presence also of disease-producing bacteria such as those causing diphtheria, typhoid and cholera. Coliform bacteria live naturally in the intestines of cows and human beings, so that if they are detected in a milk sample, it indicates that the milk has been exposed to faecal contamination and even perhaps from sewage in the water supply.

The presence of coliform bacteria in milk indicates dirty methods of production and also a lower keeping quality. If heavy coliform contamination occurs in the water-supply to the dairy, it shows that sewage is gaining access to the water at some stage. Coliform types in milk very rarely mean direct sewage contamination.

Differentiation of Faecal and Non-Faecal Types of Coliform Bacteria.

The name " coliform " covers a large group of bacteria, including types which are found predominantly in the soil and only in small numbers in the intestines, and types which are found mainly in the intestines and only in small numbers free-living in the soil. It is sometimes desired to find out if the coliform bacteria in a certain milk or water sample are of the faecal or non-faecal types. This is one of the tests carried out when investigating an outbreak of disease through the consumption of milk. All the coliform types will produce acid and gas if allowed to grow in McKonkey's Bile Salt Broth, so that they cannot be distinguished by means of that test. The true coliform bacteria belong to the *Escherichia coli* group and these are found predominantly in dung and in the intestine. The coliform types which occur mainly in the soil are known as the " atypical " coliform bacteria and they belong to the *Aerobacter* group.

There is really no very convenient single test which distinguishes the true coli or faecal types from the atypical or non-faecal types. One method is to grow the bacteria in a special medium known as " Koser citrate". This is a liquid medium where the only carbon available to the bacteria is in the form of a citrate salt; all the other ingredients are inorganic. The non-faecal types, that is the *Aerogenes* group, can grow in this medium as they can use citrate as their source of carbon. The faecal types or true coli bacteria cannot do this and, therefore, die off when put into this Koser citrate. If, then, during incubation at 37°C the Koser medium turns turbid, it means that these particular coliform bacteria are growing successfully in it and, therefore, must be of the non-faecal type.

There are also several other differentiation tests for these faecal and non-faecal types, and these include the Voges-Proskauer test, the Methyl Red test and the test for indole production.

The Fermentation Test.

This is a general test which determines roughly the type of bacterial contamination of a milk sample. It also has the advantage of being comparatively rapid as it only takes 24 hours to perform. The test simply consists of incubating about 40-50 ml. of milk in a sterile glass test-tube or boiling tube at 37°C for 24 hours and then examining the curd which has formed. Different bacteria produce different types of curd, and an approximate idea may be obtained as to the nature of the predominant bacteria in the milk under test. The following types may be obtained :—

1. No curd at all, which means that the milk is very clean and contains very few bacteria, and also that the bacteria present have very

little action upon it, as, for instance, the various micrococci which are udder commensals.

2. A firm uniform curd, which is smooth and does not exude whey, indicates a predominance of true lactic acid bacteria such as *Streptococcus lactis*. This is the normal type of bacteria to be found in milk.

3. A finely granular curd, which is smooth and lumpy in patches, shows that the milk contains not only lactic streptococci, but also numbers of organisms which cause sweet-curdling, that is, which secrete a rennin-like enzyme. These will include sporing bacilli and perhaps, *Streptococcus liquefaciens*.

4. A curd which shrinks rapidly and expels a turbid whey indicates large numbers of sweet-curdling bacteria as well as small numbers of normal lactic acid bacteria. In this case, the curd will also smell cheesy.

5. If the curd has been partially dissolved again to form a brownish-yellow liquid, then the milk contains very large numbers of casein-digesting bacteria such as the sporing bacilli.

6. A split or " blown " curd, that is, one which has been broken up by the production of gas, indicates that the milk is highly contaminated with intestinal bacteria such as those belonging to the coliform group. It can also mean that the milk has been contaminated withd ung or with sewage-polluted water.

The fermentation test is now restricted mainly to milk for cheese-making. For this purpose, the last type of curd, and contamination, is the most undesirable. The test is not at all accurate especially if the bacteria are in the milk in small numbers.

Other information which may be gleaned from the type of curd obtained during this test is the presence of mastitis bacteria, such as *Streptococcus agalactiae*. If a yellowish sediment is deposited at the bottom of the tube this usually indicates that the milk has come from a cow suffering from mastitis.

Test for the Presence of Faecal Streptococci.

This test is very useful to indicate the pollution of a dairy water-supply from sewage or other faecal sources. It is very seldom that milk is subjected to this test because the faecal streptococci, which are essentially intestinal in nature, die out very rapidly as soon as they leave the body. Thus milk will not contain living *Streptococcus faecalis* unless the sewage contamination has been exceptionally recent. Even in the case of water, this test will only show up very recent sewage contamination due to the short life these faecal streptococci have away from the intestine.

Thus polluted water often does not give a positive reaction to this test for faecal streptococci, and it can only be used in association with other tests for sewage pollution, such as the coliform test and especially the coliform differentiation test for faecal coli types. Nevertheless, if faecal streptococci are detected in a water sample it means

that the water has been contaminated with sewage very recently and if it contains any pathogenic bacteria, these too will be very active and dangerous.

The procedure of the test is as follows :—

About 0.5 ml. of the water sample is inoculated into a tube of glucose broth medium. Glucose broth is used, as faecal streptococci grow very readily in it. The tube is now incubated at 37°C for 24 hours and, at the end of that period, the deposit which forms in the tube is examined under the microscope. Any streptococci detected, that is, any strings of spherical cells, will be of the faecal type and their presence will be a sure indication of very recent contamination of the water from faeces or sewage. The degree of contamination of a water sample with faecal streptococci may be determined by inoculating various quantities of water or its dilutions and then expressing the result as for a coliform test.

The Plate Count and its Modifications.

This is a test which determines the numbers of bacteria in a milk or water sample. The bacteria, in an accurately measured small quantity of milk or water, such as 1 ml., 1/10th ml., or 1/100th ml. are encouraged to grow into colonies visible to the naked eye by being placed on a suitable solid medium which provides them with an ample supply of food. The plate count, however, is far from accurate for the following reasons :—

1. No one type of medium is suitable for the growth of all bacteria and, therefore, although the medium used may suit most of the common bacteria found in milk, there will be some which will not grow on it. This will give too low a result.

2. No one temperature of incubation will suit all the bacteria as some may be intestinal in nature and require higher temperatures for growth than, for instance, soil bacteria or lactic acid bacteria.

3. With the plate count, it is assumed that each colony of bacteria arises from a single bacterial cell, but bacteria frequently exist in clumps in milk and such a clump will only give rise to a single colony. Therefore, if many bacteria in a milk sample are grouped together in clumps, the apparent bacterial count of the milk will be low.

In spite, however, of these three faults, this test does provide a rough idea of the number of living bacteria in the milk or water, especially if the medium used is Yeastral Milk Agar and the incubation temperature is 37°C when most of the normal and also most of the undesirable milk bacteria will grow readily.

Yeastral milk agar is prepared to the following recipe :—

Agar	15 grams.
Peptone	10 grams.
Yeastral (yeast extract)	10 grams.
Sodium chloride	5 grams.
Water	1,000 millilitres

The solution is sterilised in an autoclave and then poured and allowed to set in tubes ready for use.

The milk sample is drawn aseptically with a sterile dipper and bottle. Before it is tested, it is usually made up into various dilutions as in the accompanying diagram.

METHOD OF PREPARING DILUTIONS FOR THE PLATE COUNT

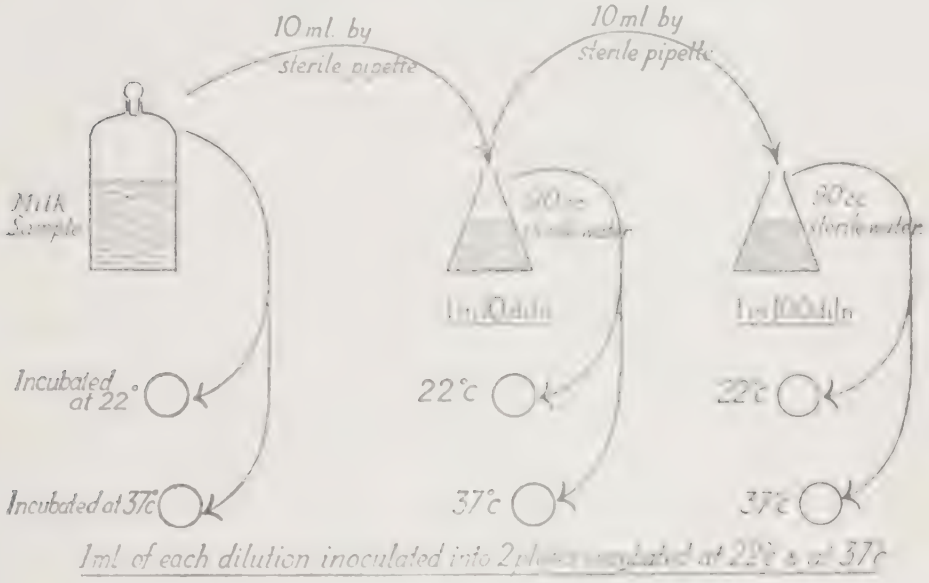


Fig. 47.

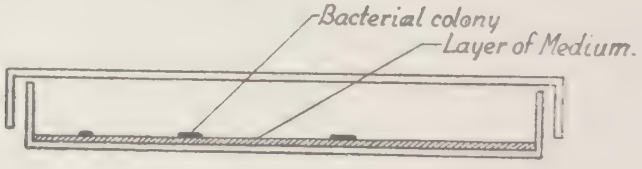
With milk and water, commonly-used dilutions are from 1 in 10 up to 1 in 1,000,000. The reason for using dilutions is to reduce the number of colonies of bacteria which will appear on any one plate. This greatly facilitates counting of the colonies.

Inoculating a Petri dish for a Plate Count.

One ml. of each dilution to be used is transferred by sterile pipette into a sterile Petri-dish.

Diagrams of a Petri Dish

View of Petri-dish showing colonies of bacteria as they appear in a plate-count.



Section showing structure of Petri-dish.

Fig. 48.

The quantity of agar medium which is required for one plate is equal to about three inches in a standard test-tube. This quantity is usually put into tubes as soon as it has been sterilised. A tubeful of the medium is liquefied by heating and poured into the Petri dish on top of the 1 ml. of milk or its dilution already there. It should not be so hot, however, as to kill the bacteria.

The plates are incubated for 48 hours at 37° C after which time the colonies which have developed are counted. Knowing the number of colonies and the dilution ratio used, it is a simple matter to calculate the number of bacteria per millilitre in the original milk or water sample.

Sometimes it is desired to differentiate between intestinal bacteria and those such as souring and soil bacteria which grow better at lower temperatures. This can be done conveniently by incubating two plates of each dilution, one at 37° C for the intestinal types and the other at 22° C for the remainder. This is the routine method when carrying out a plate count of a water sample. The Plate Count was used as one of the routine tests to check the quality of Graded milks in England and Wales. It has been superseded by the methylene blue

test, but it is still used in conjunction with a methylene blue test for Pasteurised grades of milk in England and Wales. It is also used to test certain grades of milk in Scotland, including Certified, Tuberculin Tested, Tuberculin Tested (Pasteurised) and Standard.

Several modifications of the Plate Count have been developed and these are as follows :—

Burri Smear Method. In this case, " slopes " of Yeastral milk agar in test-tubes are used instead of Petri dish plates. Only a very small volume of milk is required for each " slope". Loops of platinum wire standardised to hold .001 ml. of milk are used. Several slopes of agar are prepared by holding the test-tubes at an incline until the hot agar has cooled and solidified. This may be done as shown in *Fig.* 49. The slopes should be dried before use by keeping them at 37 C for several days.

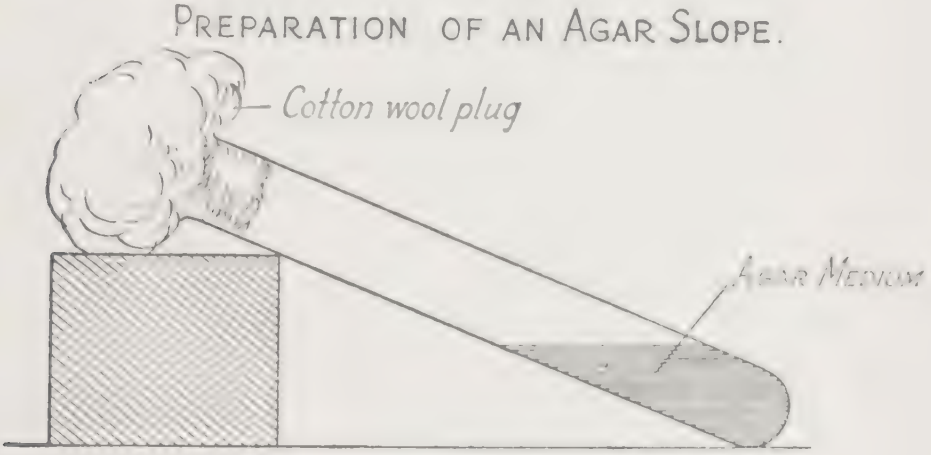


Fig 49.

Five loopfuls of the well-mixed sample are smeared on to an agar slope. The .005 ml. of milk thus added should be spread evenly and completely over the full surface area of the slope and the platinum loop should be stroked over the surface of a second slope before being sterilised. The slopes are incubated at 37 C for 48 hours and any colonies which have developed on them are counted with the aid of a lens. As the original volume of milk used was five loopfuls or .005 ml., the number of bacteria in the milk can be calculated as the number of colonies on the two slopes x 200. With dirty milks, dilutions may have to be prepared, as in the case of the Plate Count.

Advantages of the Burri Smear method include its simplicity and also the fact that it may be performed in dairies and cowsheds and thus proves useful when cases of bacterial faults are being investigated on the farm. It is, however, not as accurate as the Plate Count; this is due principally to variations in the sizes of the platinum loops employed. It is sufficiently accurate and reliable to be of value as a simple test in the tracing of sources of bacterial contamination of a milk supply. A low result may be obtained due to the bacteria

failing to grow on the relatively dry agar slope as well as when they are embedded in agar as in the case of the Plate Count. Further, certain lactic streptococci frequently fail to develop into colonies on a Burri Smear slope while others, which never appear on a Petri dish plate, do so vigorously.

The Little Plate method. The value of this modification lies in its simplicity and rapidity: 0.1 ml. of milk and 0.05 ml. of warm Yeastral milk agar are mixed together on an area 20 mm. x 25 mm. on a glass microscopic slide. After the agar has solidified, the slide is incubated at 28°C in a moist atmosphere for 24 hours, after which it is dried and the tiny colonies which have developed are stained to render them easily visible and then they are counted under a low-powered microscope. The number of colonies is counted in several different microscope fields and the average taken. Knowing the area of the microscope field and, therefore, the number of times it will divide into the area the milk was spread over on the slide, the number of bacterial colonies per .1 ml. of milk can be calculated and on multiplying by 10, the number of bacteria in 1 ml. of the milk sample can be arrived at.

With clean milks, this method can give results equally as accurate as the Plate Count, but for other milks, as such a small quantity of milk is used, the results cannot be relied on to the same extent. Moreover, all the disadvantages of the one medium and one incubation temperature applying to the Plate Count apply equally well to the Little Plate.

The Direct Microscopic Count by Breed's Smear.

This test determines the number of bacteria in a sample of milk by actually counting each cell under the microscope. It is therefore capable of giving a very accurate result. The technique of the test is as follows:—

.01 ml. of the milk sample is transferred by pipette on to an area of 1 cm. square on a glass slide. The milk is evenly spread over the square and dried by slight heat application. The slide is then immersed in Newman's stain for several minutes. This stain contains methylene blue which stains the bacteria blue and tetrachlorethane which dissolves away the fat globules thus rendering the stained bacteria easier to see. After the slide has been stained, washed and dried, it is examined under a high-powered microscope and the bacteria counted. The average number of bacteria in several, and, at least five, microscope fields is determined. Care has to be taken to differentiate between bacterial cells and leucocytes or white blood corpuscles. The latter have nuclei whereas bacteria have none.

Knowing the area of the microscope field and the ratio it bears to 1 square cm., the number of bacteria in .01 ml. of milk may be calculated and so also the number of bacteria in 1 ml. of milk. For example, if the area of the microscope field was 0.000154 square cms., that is, a diameter of 0.014 cms. then the number of fields to 1 square

cm. is 6,500. If the average number of bacteria over several fields was 2, then the number of bacteria per square cm. and therefore in 0.01 ml. of milk would be 2 x 6,500 = 13,000. Therefore, it follows that the number of bacteria per millilitre will be 1,300,000.

Making a direct microscopic count of bacteria with a Breed's Smear.

This method is very useful on account of its rapidity and the slide forms a permanent record of the bacteria in the sample. Its main disadvantage, however, is that it cannot differentiate between living and dead bacterial cells and is therefore useless for the testing of pasteurised milks. In such milks only those bacteria which are still alive after the heat-treatment and which are, therefore, liable to cause developing faults, are important. These should be relatively few in number but the count by Breed's Smear method will, by including all the cells killed off by the heat treatment, give a high result. It is also probable that the small volume of milk used does not represent the true quality of the whole bulk of the sample so that the result, while being accurate in itself, will not be accurate for the whole sample. Due to the inclusion of dead cells, the count by this method is sometimes up to four times that obtained by the Plate Count.

In Britain, this method is not used officially for milk but it is a common procedure in the United States of America. It also provides a very useful test for mastitis. Its use in this connection is described later.

Tests for Mastitis.

The disease of the udders of dairy cows known as mastitis is a very serious source of loss to the dairying industry. It is estimated that 40 million gallons of milk and £3,000,000 are lost annually due to the effects of this disease. There are several tests which can be applied to milk samples and which will permit the detection of mastitis in the cow from which the sample has been drawn. These vary a great

deal in their basic principles, but the commonest and perhaps most reliable are as follows :—

(1) *The microscopic count of leucocytes* by Breed's Smear method. This test is carried out exactly as for a bacterial count by this method. It is based on the fact that milk from a cow suffering from mastitis contains a large number of leucocytes, a high proportion of which are polymorphonuclear, that is, they contain several nuclei. The leucocytes are counted in exactly the same manner as bacteria. care being taken not to confuse the bacteria, which have no nuclei, with the white blood cells, which have. The calculation of the number of leucocytes per millilitre of milk is also similar to that for bacteria. If the milk sample contains more than 300,000 leucocytes per ml., and especially if over 75% of these are polymorphonuclear, then the cow from which the sample was drawn may be considered as a suspect case of mastitis. This test can be carried out on a mixed quarter sample, that is, a sample containing milk from all four quarters of the udder, or it may be performed with samples from single quarters. If one quarter contains a much larger number of leucocytes than the other three, then it is safe to assume that such a quarter is abnormal and is suffering from mastitis. This test does not apply to colostrum or late lactation milk, as these two normally contain large numbers of leucocytes.

(2) *The cellular content of the milk* gives an indication of the health of the cow. This is the quantity of cellular material, white blood cells and bacteria, that the milk contains. This material may be centrifuged out of the milk and its volume measured in special centrifuge tubes. Healthy cows should give milk containing not more than .1 ml. of cellular matter per 10 ml. of milk. If the milk contains more than this and especially if the sediment contains a large proportion of polymorphonuclear leucocytes, then the cow is suffering from mastitis.

(3) Milk from a cow suffering from mastitis contains a reduced percentage of lactose due to the reduced synthesising power of the diseased milk-secreting tissue. This lack of lactose would lower the osmotic pressure of the milk, but the latter must always equal that of the blood. In order therefore to maintain the osmotic pressure of the milk, the cow secretes more chloride into her milk and this in turn produces an increase in the electrical conductivity of the milk. It is a relatively simple matter to measure the conductivity of a milk sample and there are special instruments now available specially designed for this purpose. The accompanying illustration shows such an instrument being used. As the conductivity of the milk of cows has a wide natural variation, the actual figures obtained from the test have little real significance. The value of the electrical conductivity test on milk as a criterion of mastitis lies in the comparison of conductivity figures for all four quarters of a cow. Milk from an abnormal quarter has a much higher conductivity than that from the other three quarters. The operation of this test is now simple and rapid and it may be carried out in the cowshed. It is being increasingly used for the routine testing of dairy herds at fortnightly or monthly intervals for the detection of mastitis.

Determining the electrical conductivity of a milk sample.

(4) *The catalase test.* Leucocytes and aerobic bacteria with the exception of the lactic acid bacteria produce an enzyme called catalase. The amount of catalase in reasonably clean milk depends mainly on the number of leucocytes. The catalase content of milk can therefore be used indirectly as a criterion of mastitis. Catalase brings about the evolution of oxygen when hydrogen peroxide is added to the milk. This test is usually carried out by placing 15 ml. of milk into a 20 ml. tube graduated into millilitre sub-divisions. The tube is filled with a 1% solution of hydrogen peroxide and the contents mixed. The tube is then arranged so that surplus milk can discharge off into a beaker and is incubated at 22° C for six hours. After this incubation period the volume of oxygen which has been evolved is measured.

Freshly-drawn milk from a healthy cow should not produce more than 1.5 ml. of oxygen per 15 ml. of milk. If more than this is evolved, the milk is abnormal and the cow may be suffering from mastitis. No definite conclusions may be drawn from this test alone, however, as there are several other types of milk which will give a high catalase result. These are :—

(1) Colostrum, due to its high leucocyte content.

(2) Late lactation milk.

(3) Very dirty milk containing large numbers of catalase-producing bacteria.

(4) Cream, due to the large numbers of bacteria adhering to the fat globules.

The catalase test can also be used as a rough check of the efficiency of pasteurisation. Heat-treatment should kill all leucocytes and catalase-producing bacteria and also destroy any natural catalase in the milk. Properly pasteurised milk should thus evolve little or no oxygen when mixed with a solution of hydrogen peroxide. The catalase test for efficiency of pasteurisation is not nearly so reliable as the officially recognised phosphatase test.

(5) *Isolation of Mastitis-producing Bacteria* from the milk can also be used to detect mastitis in the dairy cow. The isolation may be done by inoculating 1 ml. volumes of the sample on to Petri dishes containing a medium known as Crystal Violet Blood Agar. The dye crystal violet prevents the growth of staphylococci on the plates. These would mask the colonies of pathogenic bacteria. *Streptococcus agalactiae*, the mastitis organism, has the power to decompose and render colourless the haemoglobin (the red pigment) of blood; that is, these bacteria are "haemolytic." If then, on incubation at 37°C, colonies of bacteria appear, which render the blood agar clear and so form a "halo" round themselves, and further, if microscopic examination of such colonies shows them to be streptococci, then it may be assumed that the cow is suffering from mastitis.

In addition to the above five milk tests for mastitis, various chemical indicators can be used to determine approximately the degree of acidity of the milk. These liquids change colour at various acidities and alkalinities. Papers, similar to laboratory litmus papers, may be obtained impregnated with these indicators and they are usually used to test the fore-milk of cows in the cowshed. To test a quarter, a little milk is drawn from the teat on to the prepared paper whose colour is then noted and compared with a standard reference key. Milk from a quarter affected with mastitis is usually alkaline in reaction and can thus be easily detected. Indicators commonly used for this purpose include alizarin, brom-cresol-purple and brom-thymol-blue. Normal milk turns alizarin reddish-violet while alkaline milk from a diseased quarter changes it to violet blue. With sour milk, alizarin turns yellow. Brom-cresol-purple and brom-thymol-blue are grey-blue in colour with normal milk but they turn purple with alkaline milks. The use of these indicator papers together with the strip cup to determine the presence of clots in the strippings, forms a quick and easy check for the presence of udder disease in the herd. It facilitates the comparison of the health of different quarters of the same cow. Abnormal quarters show up strikingly with a marked colour change in the indicator paper when milk is added to it.

Test for the Presence of Tubercle Bacilli in Milk.

All cows which are suffering from tuberculosis do not secrete tubercle bacilli in their milk, but cows with tubercular lesions of the udder frequently do so. The absence of tubercle bacilli from milk does not, therefore, indicate that the cow is free from tuberculosis; neither does the presence of tubercle bacilli in milk necessarily indicate that the cow is suffering from the disease, because the bacteria may have been introduced to the milk through contamination from an infected person. This latter, however, is a comparatively rare occurrence. The presence of tubercle bacilli in a milk supply renders it very dangerous from the public health point of view. It is estimated that between 1,500 and 2,000 persons die each year through contracting bovine tuberculosis from milk. Most of these deaths are children. The methods of detecting tubercle bacilli in a sample of milk are as follows :—

(1) A film of the milk on a glass slide may be stained by a special method known as Zeihl-Neelson's Acid-Fast staining method and then examined under the microscope. If rod-shaped bacteria are observed in the stained preparation and they are stained red, that is, they are "acid-fast" and thus do not lose the red stain under treatment with acid, then the milk contains tubercle bacilli. These bacteria are, with certain harmless exceptions, the only micro-organisms which do not lose the red stain in this method. Frequently the milk is first centrifuged to separate out the bacteria and other solid particles and the deposit obtained after centrifuging is then stained and examined.

(2) As the first method frequently fails to detect tubercle bacilli in the milk due to their normally very small numbers and further, as the acid-fast bacteria which are observed may simply be harmless non-pathogenic types, a more reliable second method is employed. The sample of milk is centrifuged and the resultant deposit suspended in sterile water and inoculated into the peritoneal cavities of two guinea pigs. One of the animals is killed in four weeks after the inoculation and its body examined for the presence of tubercular lesions and nodules in the various organs. If no such lesions or nodules are to be found, the other guinea pig is killed two weeks later and its body also examined for the characteristic lesions. If none is discovered this time the milk contained no tubercle bacilli. If lesions are found, however, the presence of tubercle bacilli in the milk is indicated and this is usually confirmed by staining "smear" preparations from the lesions and examining them under the microscope for acid-fact, rod-shaped bacteria. The detection of these on the slide proves conclusively that the original milk sample was contaminated with *Mycobacterium tuberculosis*.

GLOSSARY

Amyl Alcohol.—One of the higher alcohols obtained from fusel oil, a by-product derived from the distillation of alcohol produced from the fermentation of potatoes. It is used in the Gerber test to dissolve the milk fat.

Anaerobic.—This term is applied to an organism which can live in the absence of free atmospheric oxygen. Frequently free oxygen is harmful to such organisms, as, for example, the clostridia.

Autolysis.—The changes which occur when an organism dies and its own digestive enzymes start to act on it. These possibly may completely disintegrate it.

Betaine.—A colourless, tasteless, crystalline substance present in sugar beet. Decomposition of this substance produces trimethylamine, which has a strong fishy taste.

Botulism.—An acute condition of poisoning due to the active toxin produced by *Clostridium botulinus*.

Buffer-substrate.—The material used in the phosphatase test for efficiency of pasteurisation and on which the phosphatase enzyme acts.

Calorie.—In human nutrition the energy value of foods is calculated in terms of units of heat. One Calorie is the heat required to increase the temperature of 1 kilogram of water by 1°C.

Carbohydrates.—Organic compounds containing only the three elements, carbon, hydrogen and oxygen. The number of atoms of hydrogen in a molecule of a carbohydrate is twice that of those of oxygen. Common carbohydrates are the sugars, starch and cellulose.

Carotene.—A yellow pigment which is converted in the liver of the animal into Vitamin A.

Centrifugal Force.—When a body is rapidly rotated in circles it tends to fly off away from the centre of the circle in which it is being rotated. The force so developed is called centrifugal force. Thus on whirling a stone attached to a string a tension develops in the string equal to the centrifugal force developed.

Centrifugal force can be used to separate liquids of different specific gravities. The heavier liquid collects at the outside of the rotating vessel and the lighter liquid at the inside. This is the principle of the mechanical separator for separating cream.

Chlorophyll.—The green colouring matter in plants. It traps and utilises the energy of sunlight, enabling the plants to make sugars from water and carbon dioxide.

Coccoid.—Resembling a coccus in shape, that is, tending to be spherical.

Colony of bacteria.—Bacteria when growing on artificial media produce visible "clumps" of cells. Such visible clumps are known as colonies and they possess physical characteristics, such as colour and shape, which are typical of the particular species of bacteria.

Colostrum.—The type of milk produced by a cow during the first few days after calving.

Commensal.—Any type of micro-organism which naturally inhabits some organ of an animal's body and yet does not produce any disease.

Curd tension.—The resistance to cutting offered by the rennet curd is called the "curd tension." It may be measured by means of a weighted knife.

Dextrose.—A sugar, also called glucose and grape sugar, present in grapes. The sugar present in the blood is dextrose.

Epidemic.—The term given to a sudden and widespread outbreak of an infectious disease.

Endo-enzyme.—An enzyme which is always contained within a bacterial cell and is never liberated into the surrounding medium until the cell dies and autolysis commences.

Enzymes.—Substances produced by living cells, but not themselves living bodies, which can bring about chemical changes without being affected by the changes. The chemical reactions in plants and animals are brought about by enzymes.

Esters.—Compounds of fatty acids with alcohols.

Fatty acids.—Animal and vegetable fats are mainly mixtures of "Glycerides." Each glyceride is a compound of glycerine (glycerol) with a fatty acid.

Flagella.—The hair-like appendages by means of which certain bacteria swim through liquids. These flagella have a whip-like action which propels the bacteria along.

Fibrin.—One of the constituents of blood mainly responsible for the clotting of blood.

Glucose.—See "Dextrose."

Glycerides.—See "Fatty acids."

Hormone.—A complex chemical substance with a pronounced and specific effect on a particular part of the body, such as the udder. Hormones are produced by the endocrine or "ductless" glands, which pour their secretions into the blood stream and they are highly active in only very minute amounts, measurable in parts per million.

Hydrogen Peroxide.—A compound of hydrogen and oxygen which acts as an oxidising and bleaching agent. Hydrogen peroxide decomposes to form oxygen gas and water.

Lecithin.—One of the so-called "phosphorised fats" which contain phosphoric acid and choline (a nitrogen compound) in addition to fatty acids and glycerol. This type of fat is much more active chemically than the "neutral fats," which contain only fatty acids and glycerol, and plays an important part in the chemical reactions in the animal body. Lecithin in milk may be decomposed to yield trimethylamine, thus producing a fishy taint.

Lesion.—An ulcer produced during the course of a disease. It may be situated in any part of the body.

Leucocyte.—A white blood corpuscle.

Lovibond Units.—The Lovibond Tintometer is an instrument for measuring colours. It contains glasses tinted with the three primary colours, red, yellow and blue. The glasses are marked in Lovibond Units, so that it is possible to define colours in units of intensity.

Mastitis.—Inflammation of the udder caused by certain species of bacteria.

Medium.—An artificial food mixture upon which bacteria are grown in the laboratory.

Microscopic field.—The circular area which is visible on looking through a microscope. Its area varies with the magnifying power of the instrument.

Milk Serum.—The residue remaining after removing the fat from milk.

Mucin.—A nitrogenous chemical substance with a slimy, adhesive nature.

Nitrifying bacteria.—These bacteria, *Nitrosomonas* and *Nitrobacter*, carry out nitrification in the soil and convert ammonium salts to nitrites and then to nitrates. Plants absorb their nitrogen from the soil in the form of nitrates.

Non-fatty Soaps.—Normal soaps are made by boiling fats with caustic soda or caustic potash. They have the disadvantage that they react chemically with hard water, producing a scum. Non-fatty soaps are without this disadvantage. They are not made from fats, but are complex organic compounds mainly derived from mineral oils.

Nucleus.—The "brain" of a cell or micro-organism. It consists of dense protoplasm and controls the activities of the cell or micro-organism. All plant and animal cells have nuclei.

Oestrogens.—A group of "sex" hormones connected with the reproductive processes of the body. Their injection into barren or virgin cows may induce lactation.

Paracasein.—The compound formed from the casein of milk by the action of the enzyme "rennin" present in rennet.

Pathogenic bacteria.—Any type of bacteria capable of causing disease in plants or animals.

Permutit.—A type of mineral used in water softening. It absorbs calcium and magnesium from the water and liberates sodium into the water. Water passed through a filter containing permutit does not react with soap.

Peritoneal cavity.—The abdominal cavity of an animal in which the digestive organs are suspended.

pH.—The "pH scale" is a scale of numbers from 0 to 14 used for indicating the intensity of acidity or alkalinity of solutions. Neutral solutions have a pH of 7. Lower figures indicate acidity and higher figures alkalinity.

Pigment.—A form of colouring matter, frequently produced by certain bacteria.

Protease.—An enzyme which can decompose proteins. Such enzymes are also called "proteolytic enzymes."

Proteolysis.—The digestion of break-down or proteins.

Proteolytic enzyme.—A complex chemical substance capable of causing proteins to split up into simpler substances.

Reductase.—An enzyme produced principally by souring bacteria and capable of reducing a dye (*i.e.*, removing oxygen from it) and changing its colour.

Riboflavin.—One of the vitamins of the "Vitamin B Complex" needed by humans and poultry. The greenish-yellow pigment present in whey is riboflavin.

Smear preparation.—A preparation in which a small amount of a bacterial colony from a plate or other culture is smeared over an area of a glass slide and is stained ready for microscopic examination.

Specific Gravity.—The ratio between the weight of unit volume of a substance and that of an equal volume of water is called the specific gravity of the substance.

Starters.—Cultures of lactic acid bacteria used in butter and cheese-making.

Substrate.—The name given to the food material upon which bacteria are feeding.

Toxin.—A chemical substance produced by some organisms and capable of causing symptoms of poisoning in humans and animals.

Trimethylamine.—A compound of intensely fishy taste and smell occurring in herring brine.

Tubercular nodule.—The later stage of a lesion caused by tuberculosis. The ulcer tissue becomes solid and "cheesy" and forms a lump or nodule.

Ultra-violet Rays.—Visible light is made up of rays of various wave lengths. Red rays have the longest and violet rays the shortest wave lengths. Sunlight contains also rays shorter than the latter but invisible to the eye. These are the ultra-violet rays and are largely responsible for sunburn of the skin. Ultra-violet rays produce Vitamin D, which prevents rickets, from substances present in the fat below the skin.

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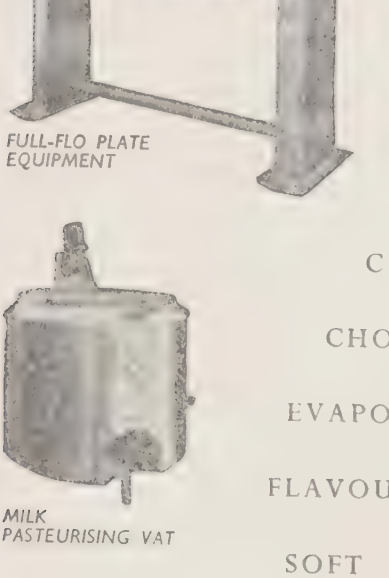

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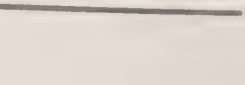

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